MAY 2 5 1929

Vol. XIV MAY, 1929 No. 8

The Journal of Laboratory and Clinical Medicine

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The Journal of Laboratory and Clinical Medicine

VOL. XIV ST. LOUIS, MO., MAY, 1929 NO. 8

CLINICAL AND EXPERIMENTAL

SYNTHALIN IN THE TREATMENT OF DIABETES*

BY ELAINE P. RALLI, M.D., AND CONNIE M. GUION, M.D., NEW YORK

IN VIEW of the widespread interest in synthalin and the scarcity of clinical reports following its use, the cases in our clinic at present under treatment are presented. In Frank, Nothmann and Wagner's original articles[1, 1a, 1b] they state that synthalin is a derivative of aminopenthylen-guanidin in which, by lengthening the methyl chain, the toxicity of the parent substance is greatly lessened. Their results, experimental and clinical, showed that synthalin caused a reduction of blood sugar in both the normal and the diabetic, but just how this oxidation of carbohydrate was effected was not explained. Other investigators who obtained similar results clinically are Joslin,[2] Carrasco,[3] Duncan,[4] and Ringer and his associates.[5]

Lewis and Izume[6] showed that hydrazene produces a hypoglycemia and that this is accompanied by an increase in the amino acids in the blood due to a failure of deaminization resulting from liver injury. Their explanation of the hypoglycemia is that the supply of glucose available for combustion is decreased because glyconeogenesis, the transformation of noncarbohydrate material to glucose, is interfered with, due to the hepatic injury produced by hydrazene. This work suggested to Blatherwick and his associates[7] that synthalin might produce its effect in a similar manner. They administered synthalin by stomach, subcutaneously and intravenously to rabbits. The doses in each case were much larger per kilogram of body weight than are used therapeutically, and it must be remembered that the subcutaneous and intravenous routes are never used clinically. In the rabbits receiving synthalin by stomach there was no effect either on the sugar or the amino acid content of the blood. The latter is taken as an index of hepatic injury. On the other hand, subcutaneous and intravenous administration produced a hypo-

*From the Diabetic Clinics of the New York Infirmary for Women.
Received for publication, July 24, 1928.

glycemia and a rise in urea and amino acid nitrogen. When synthalin was administered with glycine, there was a marked delay in the return of the amino acids of the blood to the normal level. Blatherwick and his associates, therefore, conclude that synthalin like hydrazene interferes with deamination by causing hepatic injury; that hydrazene causes a more severe injury, and that the hypoglycemia resulting from the use of synthalin may be due to this injury to the liver and a consequent interference with normal glyconeogenesis. They admit that they are not in a position to know whether or not these results would follow its oral administration in clinical diabetes, since in their experiments on rabbits in which synthalin was given by mouth there was no hypoglycemia nor any increase in the amino acid nitrogen of the blood. In one of these animals they point to a loss of body weight amounting to 0.4 kilo. in a period of ten days and a decrease in the urea nitrogen in the blood as an evidence of toxic action. It is recognized that synthalin in too large doses produces a diarrhea and gastric cramps, due probably to the guanidin radicle, and this may have accounted for the loss of weight in this animal, as the dose administered per kilogram of body weight was about 7 mg., while the average dose for a patient is 0.5 to 0.8 mg. per kilogram of body weight, and this is discontinued every third or fourth day for one day.

It is generally agreed that synthalin will lower the blood sugar in diabetic patients when given by mouth. Naturally there is great interest as to its effect on liver function, but satisfactory data are lacking on this point. The explanation of Blatherwick and his associates based on interference with glyconeogenesis due to liver injury is theoretic and not supported by the evidence of Frank and his coworkers. These found in depancreatized dogs that the blood-sugar level was reduced and the urine remained sugar and acetone free after the administration of synthalin by mouth. Such animals are, of course, unable to oxidize carbohydrate given as such. Lublin[8] has shown the effect on the respiratory quotient of the administration of glucose with and without synthalin. In the same patient, a severe diabetic, 30 gm. of glucose administered by mouth was not oxidized, the R. Q. remaining between 0.65 and 0.708. Following a day on which 40 mg. of synthalin were administered by mouth the experiment was repeated and the R. Q. was never below 0.721 and rose to 0.832. This is most significant, and points to the utilization of carbohydrate by a patient who was otherwise unable to make any use of it. The observations were repeated on several other patients with similar results, the R. Q. in one case rising to 0.967. These results cannot be explained on the basis of interference with glyconeogenesis.

A total of twelve cases have been under our observation, the longest, since July, 1927. The summary of the cases and the effect of synthalin are given. We have never felt justified in using the drug unless the patient could not remain sugar free and maintain a moderate blood-sugar level on a caloric diet compatible with the demands of her daily life. The patients usually did better while in the hospital, probably due to the regulation of the diet, which remains an important factor in the treatment of diabetes.

REPORT OF CASES

CASE 1.—M. H., female, aged forty-five, weight 162¼ pounds, height 67 inches, admitted originally to the hospital April 2, 1927. History of chronic diabetes. On admission the patient was drowsy, mentally confused and with a strong acetone odor to her breath. Blood sugar 370 mg. This patient was first treated with insulin and was discharged on April 21, on a total of 50 units daily in two divided doses, and a diet of 2100 cal. The insulin dosage

TABLE I.

CASE 1.

DATE	URINE SUGAR	URINE ACETONE	BLOOD SUGAR	INSULIN UNITS	SYNTHALIN MG.	NO. OF DAYS	DIET
Sept. 9	0	0	125	8	10	Daily	C – 100
Sept. 10	0	0		5	10		P – 60
Sept. 11	0	0		2	10		F – 150
Sept. 12	0	0		0	20		Cal. – 2100
Sept. 13	0	0		0	20		The diet was
Sept. 15	0	0	152	0	30		not changed
Sept. 29	0	0	109	0	20		at any time
					20		
Oct. 13	0	0	135	0	alternating		
					with 30		
Oct. 27	0	0	142	0	30	For 2 days off 1 day	
Nov. 2	0	0	*177	0	30	For 3 days off 1 day	
Nov. 17	Tr.	0	154	0	30	Same	
Dec. 8	0	0	145	0	30	Same	
1928							
Jan. 9	0	0	92	0	30	For 2 days off 1 day	
Feb. 16	0	0	100	0	30	Same	
Mar. 1	0	0	118	0	20	q. 2 d.	
Mar. 22	0	0	130	0	20		
Apr. 19	0	0	138	0	20		

*The patient had been constipated for several days and had had a cold.

TABLE II.

CASE 2.

DATE 1927	URINE SUGAR	URINE ACETONE	BLOOD SUGAR	SYNTHALIN MG.	NO. OF DAYS	C	P	F
July 5	Tr.	Tr.	120	0		40	40	100
July 15	Tr.	0	–	20		60	60	150
July 16	0	0	–	25			Same	
July 17	0	0	–	50			Same	
July 18	–	–	–	0			Same	
July 19	0	0	102	25	Daily		Same	
July 21	0	0	–	10	Daily		Same	
July 28	0	0	104	10	Daily		Same	
Aug. 9	0	0	138	12½	Daily		Same	
Aug. 30	0	0	130	10	Daily		Same	
Sept. 7	0	0	85	10	Daily		Same	
Sept. 20	0	0	87	10	Daily		Same	
Oct. 13	0	0	131	10	Daily		Same	
Nov. 10	0	0	107	10	Daily		Same	
Dec. 15	0	0	110	10	Daily		Same	
1928								
Jan. 5	0	0	109	10	Daily		Same	
Feb. 16	0	0	123	10	On 2 days, off 1 day			
Mar. 19	0	0	163	0	Discontinued until April 5	60	60	125
Apr. 5	+	0	180	10	Begun again daily		Same	
May 10	0	0	125	10	On 3 days, off 1 day		Same	

was reduced the second of May to 39 units daily, and on July 7, to 30 units. On the ninth of September, at which time she was receiving a daily dose of 12 units of insulin, 4 of the insulin units were replaced with 10 mg. of synthalin. On September 10, the insulin dose was decreased to 5 units, the synthalin remaining at 10 mg. On the eleventh of September the insulin was reduced to 2 units, synthalin still 10 mg., and on the next day insulin was discontinued and two 10 mg. tablets of synthalin were given. The urine during this period remained sugar free. The diet was unchanged.

CASE 2.—R. R., female, aged forty-two, weight 190 pounds, height 60 inches. Associated symptoms pruritus vulvae. This patient was sensitive to insulin. She had been admitted to the hospital for treatment on two separate occasions. The first time was in October, 1926, with a blood sugar of 214 mg., and sugar and acetone in her urine. After six weeks she was discharged on a diet of 1525 calories and 10 units of insulin daily. Her second admission was in January, 1927. Her complaint was a rash following insulin injections. This consisted of a maculopapular eruption on both arms, across the chest and back, and on her thighs. The left arm and hand were considerably swollen, and there was a good deal

TABLE III.

CASE 3.

DATE 1927	URINE SUGAR	URINE ACETONE	BLOOD SUGAR	SYNTHALIN MG.	NO. OF DAYS	DIET C	DIET P	DIET F
Oct. 18	++	+		0		Thrice cooked 5		
Oct. 19	0	0	273	0		per cent veg-		
Oct. 20	2.2	+		0		etables only were		
Oct. 21	0	+		0		allowed in this		
Oct. 22	0	+	200	0		period		
Oct. 23	0	0		0				
Oct. 24	0	0		0				
Oct. 25	0	+		0				
Oct. 26	0	+		20		50	75	110
Oct. 27	0	+		30			Same	
Oct. 28	0	+	159	45			Same	
Oct. 29	0	+		35			Same	
Oct. 30	Tr.	+		35			Same	
Oct. 31	Tr.	0	172	35			Same	
Nov. 1	0	0		25			Same	
Nov. 2	+	0					Same	
Nov. 3	+	0		45			Same	
Nov. 4	0	0		45			Same	
Nov. 5	0	0					Same	
Nov. 6	0	0		45			Same	
Nov. 7	0	0	153	45			Same	
			Discharged from hospital and treated in clinic					
Nov. 17	0	0	222	35	On 3 days, off 1 day		Same	
Dec. 1	++	Tr.	215	55	On 3 days, off 1 day		Same	
Dec. 8	++	Tr.	185	45	On 3 days, off 1 day		Same	
Dec. 15	++	Tr.	179	45	On 2 days, off 1 day		Same	
Dec. 22	++	Tr.	212	45	On 3 days, off 1 day	60	60	110
1928								
Jan. 5	++	Tr.	127	25	On 2 days, off 1 day		Same	
Jan. 19	Tr.	0	227	35	On 3 days, off 1 day		Same	
Jan. 26	0	0	190	45	On 3 days, off 1 day		Same	
Feb. 2	0	0	105	25	On 3 days, off 1 day		Same	
Feb. 9	0	0	177	45	On 2 days, off 1 day		Same	
Feb. 16	Tr.	0	202	45	On 3 days, off 1 day		Same	
Mar. 15	Tr.	0	215	45	On 3 days, off 1 day		Same	
Mar. 29	Tr.	0	168	45	On 3 days, off 1 day		Same	
Apr. 12	0	0	168	45	On 3 days, off 1 day		Same	
Apr. 26	Tr.	0	181	45	On 3 days, off 1 day		Same	
May 10	Tr.	0	194	45	On 3 days, off 1 day		Same	

of itching. Her blood sugar was 103 mg., which rose to 136 mg. after breakfast. Skin tests showed the patient sensitive to insulin. She was discharged on a diet of 1000 calories and a blood sugar of 77 mg. Her weight at this time was 156 pounds. After discharge the patient was followed in the clinic. Her blood sugar varied from 120 mg. to 150 mg., and there were traces of sugar in her urine. On July 5, the patient was prepared for synthalin treatment, the diet being increased to 1220 calories, consisting of C : 40, P : 40, F : 100. The following chart will show her progress and includes the period when the drug was stopped for a period of time, at which time her blood sugar rose and sugar reappeared in the urine.

CASE 3.—F. M., female, aged fifty-seven, weight 181½ pounds, height 60 inches. History of chronic diabetes. Admitted to the hospital for treatment of increasing weakness and pains in the legs.

TABLE IV.

CASE 4.

DATE 1927	URINE SUGAR	URINE ACETONE	BLOOD SUGAR	SYNTHALIN MG.		DIET C	DIET P	DIET F	BLOOD PRESSURE
Jul. 28	+	Tr.	200	0		40	40	100	190/100
Jul. 29	+	Tr.		0		The diet remained			
Jul. 30	+	Tr.		0		unchanged			
Jul. 31	+	Tr.		0					
Aug. 1	+	Tr.	265	0					135/ 80
Aug. 2	+	Tr.		0					135/ 80
Aug. 3	+	Tr.		0					135/ 80
Aug. 4	+	Tr.		0					135/ 80
Aug. 5	+	Tr.		0					135/ 80
Aug. 6	+	Tr.		0					135/ 80
Aug. 7	+	Tr.		0					135/ 80
Aug. 8	+	Tr.		25					132/ 80
Aug. 9	0	0	208	25					130/ 65
Aug. 10	0	0		25					150/ 80
Aug. 11	0	0		25					140/ 72
Aug. 12	0	0	174	25					145/ 65
Aug. 13	0	0		25					128/ 75
Aug. 14	0	0		25					135/ 78
Aug. 15	0	0	161	25					142/ 70
Aug. 16	0	0		25					130/ 70
Aug. 17	Tr.	Tr.		25					142/ 78
Aug. 18	0	0		25					130/ 70
Aug. 19	0	0		25					134/ 74
Aug. 20	0	0		25					120/ 65
Aug. 21	0	0		25					120/ 70
Aug. 22	0	0	126	25					120/ 80
Aug. 23	0	0		25					120/ 80
Aug. 24	0	0	112	25					110/ 65
Aug. 25	0	0		25					110/ 65
Aug. 26	0	0	147	25					110/ 65
Sept. 22	0	0	98	25	Daily				130/ 80
Sept. 29	0	0	105	25	Daily				116/ 70
Oct. 11	0	0	134	25	Daily				148/ 90
Oct. 20	0	0		25	Daily				115/ 70
Oct. 27	0	0	113	25	Daily				
Nov. 10	0	0		25	Daily				
Nov. 17	0	0	121	25	Daily				
Dec. 1	0	0	178		None since Nov. 25				150/100
Dec. 8	0	0	96	25	Daily				
1928									
Feb. 16	0	0	142		None since Dec. 27 Cardiac condition poor Patient not cooperating				170/100
Mar. 22	0		200		Patient in failure Entered hospital				

TABLE IV.

CASE 4.—CONTINUED

DATE 1928	URINE SUGAR	URINE ACETONE	BLOOD SUGAR	SYNTHALIN MG.	INSULIN (UNITS)	DIET C	DIET P	DIET F	BLOOD PRESSURE
Mar. 23	0	+	197	0	8	100	50	70	148/ 90
Mar. 24	0	+		0	8		Same		
Mar. 25	0	0		0	8		Same		
Mar. 26	0	0	190	0	10		Same		
Mar. 27	0	0		0	10		Same		138/ 76
Mar. 28	0	0	178	0	10		Same		
Mar. 29	0	0		0	10		Same		
Mar. 30	0	0	178	0	10		Same		
Apr. 1	0	0		0	0	52	32	66	
Apr. 2	0	0	178	0	0		Same		
Apr. 3	0	0		0	0		Same		128 /70
Apr. 4	0	0	195	0	5		Same		
Apr. 5	0	0		0	5		Same		
Apr. 6	0	0	170	0	5		Same		
Apr. 7	0	0		0	5		Same		
Apr. 8	0	9		0	5		Same		
Apr. 9	0	0	178	0	5		Same		
Apr. 10	0	0		25	0	40	40	80	
Apr. 11	0	0	139	25	0		Same		
Apr. 12	0	0		25	0		Same		
Apr. 13	0	0	130	25	0		Same		120/ 70
Apr. 14	0	0		25	0		Same		
Apr. 15	0	0		0	0		Same		
Apr. 16	0	0		25	0		Same		
May 3	0	0	138	25	Daily 0	50	50	100	130/ 80

CASE 4.—M. W., female, aged fifty-one, weight 168½ pounds, height 56 inches. Diabetes since 1925. This patient has auricular fibrillation and a tendency to hypertension which is especially marked when she has a high blood sugar. She was admitted to the hospital on July 28, 1927, with signs of cardiac failure, pruritis and a blood pressure of 190/100. She was digitalized, and has been on digitalis ever since.

TABLE V.

CASE 5.

DATE	URINE SUGAR	URINE ACETONE	BLOOD SUGAR	SYNTHALIN MG.	NO. OF DAYS	DIET C	DIET P	DIET F	CALORIES
Sept. 22	+ + + +	+ + +		0		5% vegetables			600
Sept. 29	+ + +	+	308	30	Daily	33	33	68	900
Oct. 4	0	0	130	10	Daily	33	33	68	900
Oct. 14	0	0	134	0	None	35	35	80	1024
Oct. 27	0	0	171	10	Daily	35	35	80	1024
Nov. 3	0	0	185	20	Daily	35	35	80	1024
Nov. 10	0	0	158	20	On three days, off one day	35	35	80	1024
Nov. 17	0	0	135	20	On two days, off one day	40	40	100	1220
Dec. 1	0	0	116	10	On three days, off one day	50	50	125	1525
Dec. 15	0	0	123	10	On three days, off one day	50	50	125	1525
Dec. 29	0	0	94	10	On three days, off one day	50	50	125	1525
1928									
Jan. 19	0	0	100	None since Jan. 5		50	50	125	1525
Feb. 8	0	0	120	0		50	50	125	1525
Mar. 1	0	0	148	0		50	50	125	1525
Mar. 22	0	0	165	0		50	50	125	1525
May 17	0	0	206	0		Has not adhered to diet			

CASE 5.—B. K., female, aged forty-eight, weight 143 pounds, height 61 inches. Has known she had diabetes for about one year. Lately has felt dizzy and weak. Pruritis vulvae, severe headache, thirst and polyuria. On admission to the clinic on Sept. 22 the patient appeared dopey, slight odor of acetone on the breath, and tongue reddened. The patient was advised to enter the hospital but refused. She was put on a diet consisting of 5 per cent vegetables and two oranges daily, and sent in for a blood sugar.

TABLE VI.

CASE 6.

DATE	URINE DIACETIC	URINE ACETONE	URINE SUGAR	BLOOD SUGAR	SYNTHALIN MG.	NO. OF DAYS	DIET C	DIET P	DIET F	CALORIES
1927										
Dec. 20	+	+	++		0		66	24	37	693
Dec. 21	+	+	++	225	0		66	24	37	693
Dec. 22	+	+	.06	154	0		66	24	37	693
Dec. 23	+	+	.04		0		66	24	37	693
Dec. 24	+	+	.04		0		66	24	37	693
Dec. 25	+	0	0		0		75	25	35	715
Dec. 26	0	0	0		0		75	25	35	715
Dec. 27	0	0	.03	155	30	Daily	75	25	35	715
Dec. 28	0	0	0		30	Daily	101	35	43	931
Dec. 29	0	0	0		55	Daily	101	35	43	931
Dec. 30	0	0	0	150	0	None	101	41	48	1000
1928				Clinic Record						
Jan. 5	0	0	Tr.		30	For 3 days	100	45	60	
Jan. 12	0	0	+	139	30	For 3 days	100	45	80	
Jan. 19	0	0	+	125	30	For 3 days	100	45	80	
Jan. 26	0	0	+	177	30	For 3 days	100	45	80	
Jan. 31	0	0	0	150	30	For 2 days	100	45	80	
Feb. 16	0	0	0	151	30	For 2 days	100	45	80	

TABLE VII.

CASE 7.

DATE	URINE DIACETIC	URINE ACETONE	URINE SUGAR	BLOOD SUGAR	INSULIN UNITS	SYNTHALIN MG.	C	P	F	CALORIES
1927										
Oct. 25	0	+	+++			0	65	40	80	1140
Oct. 26	0	+	++			0		Same		
Oct. 27	0	+	+	150	10	0		Same		
Oct. 28	0	+	+		10	0		Same		
Oct. 29	0	+	+		20	0		Same		
Oct. 30	+	+	Tr.	166	10	0		Same		
Oct. 31	0	0	Tr.		10	0		Same		
Nov. 1	0	0	Tr.		10	0		Same		
Nov. 2	0	+	Tr.		10	0		Same		
Nov. 3	0	+	Tr.		10	0	75	45	90	1290
Nov. 4	0	+	0		10	0		Same		
Nov. 5	0	+	0	217	15	0				
Nov. 6	0	+	0		40	0				
Nov. 7	0	0	Tr.	166	20	0				
Nov. 8	0	0	Tr.		20	0	75	50	110	
Nov. 9	0	+	Tr.		25	0		Same		
Nov. 10	0	0	0	250	25	0		Same		
Nov. 11	0	0	0		40	0		Same		
Nov. 12	0	0	Tr.	194	10	10		Same		
Nov. 13	0	0	0		20	10		Same		
Nov. 14	0	0	0	154	10	20		Same		
Nov. 15	+	+	0		10	20		Same		
Nov. 16	+	0	0	195	10	20		Same		
Nov. 17	+	0	0		5	30		Same		

CASE 6.—R. H., female, aged 39 years, weight 143 pounds, height 60 inches. Diabetes since 1925. Complaints pruritis vulvae, polyuria and polydipsia. Physical essentially negative. Admitted to the hospital for observation.

Patient jaundiced. Entered hospital, where she remained ten days and was discharged on diet alone and with a blood sugar of 100 mg. due to the starvation following two successive days of gall bladder drainage. She went away to the country for two months and changed her diet to what she thought was right, and a blood sugar of 212 mg. She refused insulin and has not returned to the clinic.

CASE 7.—M. G., female, aged fifty-nine, weight 160 pounds, height 64 inches. Admitted to the hospital for an infected finger. Diabetic for about ten years.

This patient only returned to the clinic once. She was sugar free on synthalin alone, and her blood sugar was 150 mg.

CASE 8.—F. L., female, aged twenty-nine, weight 152 pounds, height 63¼ inches. Admitted to the hospital Nov. 23, 1927, complaining of polyuria, polydipsia, and extreme nervousness.

TABLE VIII.

CASE 8.

DATE	URINE DIACETIC	URINE ACETONE	PER CENT SUGAR	BLOOD SUGAR	SYNTHALIN MG.	NO. OF DAYS	DIET C	DIET P	DIET F	CALORIES
1927										
Nov. 23	0	0	.5	227	0		101	35	43	931
Nov. 24	0	0	.4		0		66	24	37	693
Nov. 25	0	0	0	185	0		34	15	30	466
Nov. 26	0	0	0	206	0		22	19	37	497
Nov. 27	0	0	0		0			Same		
Nov. 28	0	0	0		0			Same		
Nov. 29	0	0	0		0			Same		
Nov. 30	0	0	0		0			Same		
Dec. 1	0	0	0		0		32	24	37	557
Dec. 2	0	++	.09	133	0			Same		
Dec. 3	0	0	0		0			Same		
Dec. 4	0	0	0		0			Same		
Dec. 5	0	0	.07		0		42	29	52	752
Dec. 6	0	0	.01		0			Same		
Dec. 7	0	0	.01		30		52	32	66	930
Dec. 8	0	0	.01	116	30			Same		
Dec. 9	0	0	.01		30			Same		
Dec. 10	0	0	0		0			Same		
Dec. 11	0	0	0		45			Same		
Dec. 12	0	0	0	124	30		64	44	83	930
Dec. 13	0	0	0	94	45		74	52	88	1296
Dec. 14	0	0	0	100	45		84	61	94	1426
Dec. 15	0	0	0	99	45		98	65	106	1606
Dec. 16	0	0	0		10			Same		
Dec. 17	0	0	0		30			Same		
Dec. 27	0	0	0	121	30	On 3 days, off 1 day	98	65	106	
1928										
Jan. 1	0	0	0	113	20	On 2 days, off 1 day				

Patient failed to return to clinic.

CASE 9.—C. V., female, aged sixty-two, weight 118 pounds, height 61 inches. Diabetic since 1922. The patient was admitted to the hospital Dec. 18, 1927, complaining of dizziness and pains in the legs and weakness for the past 2 to 3 weeks. She looked acutely ill, was slightly dyspneic, and there were some râles at the base of the right lung. The heart was negative. Knee jerks absent. Positive history of syphilis.

TABLE IX.

CASE 9.

DATE	URINE DIACETIC	URINE ACETONE	URINE SUGAR	BLOOD SUGAR	INSULIN UNITS	SYNTHALIN MG.	NO. OF DAYS	DIET C	DIET P	DIET F	DIET CAL.
1927											
Dec. 18	+	+	++++			0		80	20	40	760
Dec. 19	+	+	.08	251	20	0			Same		
Dec. 20	0	0	0	138	25	0		110	30	40	920
Dec. 21	+	+	0	222	35	0		108	35	70	1200
Dec. 22	0	0	0	163	35	0			Same		
Dec. 23	0	0	0		35	0			Same		
Dec. 24	0	0	0	140	25	0			Same		
Dec. 25	0	0	0		20	0			Same		
Dec. 26	0	0	0		20	0			Same		
Dec. 27	0	0	0	156	20	0			Same		
Dec. 28	0	0	0		15	30			Same		
Dec. 29	0	0	0		5	30			Same		
Dec. 30	0	0	0	140	5	30			Same		
Dec. 31	0	0	0		0	45			Same		
1928				Clinic Record							
Jan. 12	0	0	0	98	0	20	q. 2 days	108	35	80	
Jan. 19	0	0	0	121	0	20	q. 2 days		Same		
Feb. 2	0	0	0	168	0	30	q. 2 days		Same		
Feb. 9	0	0	0		0	30	q. 2 days		Same		
Feb. 16	0	0	0	142	0	30	q. 2 days		Same		

DISCUSSION

Of the twelve cases treated all show a definite fall in blood sugar, some more marked than others. The urine in each case remained sugar free. We might justly be criticized because in several cases the carbohydrate content of the diet was relatively low, and the patient on a fairly low caloric intake. These patients, however, showed no loss in body weight, and when the synthalin was stopped the blood sugar rose and sugar reappeared in the urine. In Case 5, the patient can now remain sugar free on diet alone, but originally when synthalin was withdrawn the blood sugar rose from 134 mg. to 171 mg., and remained at this level until the patient was "synthalinized" again, and on continued treatment went as low as 94 mg. This was equally true in other cases following withdrawal.

The question of the mode of action of synthalin remains of paramount importance. Up to this point only one of the patients observed showed any toxic symptoms referable to the liver. This was Case 6, who after two months of treatment was slightly jaundiced. She reentered the hospital for observation and the drug was discontinued. The jaundice disappeared in about four days. No satisfactory information could be obtained as to liver function.

Urobilinogen tests on the urine have been negative in all but one case, and in that case it was only faintly positive.

One cannot help but feel that synthalin is a step forward in the treatment of diabetes, as it marks the inception of a drug which given by mouth has a definite effect on the blood-sugar level. In its present form, and until its toxicity is definitely established, it should be used with care and discrimination. We do not agree with Duncan[4] that it should be used in every case of diabetes. It cannot and should not replace insulin, especially in the

younger diabetics. We have used it in cases which we could not keep sugar free on diet alone and who were unwilling to take insulin.

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ANAPHYLAXIS IN HEMOPHILIA*

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HEMOPHILIA has been the subject of much research with respect to its etiology and treatment; and it is not unnatural that many theories should be evolved toward explaining its nature. The disease is as old as recorded history itself, but seems still to defy the most energetic methods directed toward the investigation of its cause. In 1921, Vines[1] accidentally noticed a marked reduction in the clotting time of the blood in a hemophiliac who was receiving serum subcutaneously as a form of treatment. In following this case he was impressed with the possibility of controlling the bleeding by induced anaphylaxis, and suggested its trial more as a field for further research than as a specific cure of the disease.

Vines has reported his results with the use of anaphylaxis in three cases of true hemophilia. The method employed consisted of injecting 4 c.c. of normal horse serum subcutaneously, and then in eight to fourteen days repeating the injections with a much smaller amount (0.2 c.c.), and noting the presence of a wheal. In analyzing these results one is impressed by the fact that the bleeding time in each case varied considerably, and that bleeding episodes continued to occur during the treatment. The first case in which this method was carried out was followed over a period of four and one-half months during which time the patient remained sensitive to horse or sheep serum as shown by intradermal tests. In this case it was noted that there was a reduction in the clotting time immediately after the intradermal injection of serum but that bleeding episodes occurred following the injection, regardless of the degree of sensitization. However, Vines considers that a clinical cure of hemophilia had been effected, over a period of twenty-nine days, in spite of the appearance of subcutaneous ecchymoses, which he suggests might be due to "an increased fragility of the vascular walls."

The second case which Vines studied was followed over a period of

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Received for publication, September 19, 1928.

four months. In this case he noticed that the clotting time was quite low immediately following a hemorrhage but varied considerably during the time the patient was sensitive to serum. The prothrombin quotients $\frac{\text{(prothrombin time of patient)}}{\text{(prothrombin time of control)}}$ varied in this case between 0.7 and 2.4, but he believed that the clotting time remained normal for more than forty days, and there were no bleeding episodes noted during this time. In this case, then, there is a remarkable retrogression of hemophiliac accidents over a period of four months, during which the clotting time of the blood was estimated at weekly intervals.

The third case was followed for one month during which the prothrombin quotient varied between 2.5 and 8.0. In this case, four intradermal injections were given after the preliminary dose of 7 c.c. of normal sheep serum. The coagulation ratio varied between 1.2 and 8.0 and the results were further confused by the necessity of transfusing the father's blood to the patient. Vines considers the case as unsuccessful, although he states that immediately after each injection there was an acceleration of the coagulation rate.

A differentiation seemingly must be made among the possible effects from the serum itself, the induced anaphylactic state, and the reactions which must have occurred due to the injection of foreign proteins. In commenting on these objections, Vines quotes abundant evidence from other workers which would make it appear that usually simple serum injections are not satisfactory in the treatment of hemophilia, and that the danger of anaphylactic shock would be imminent in certain cases. He further quotes Sahli on the point that immediately after, and for a period following hemophyliac hemorrhages, the blood coagulation may be shorter than normal.

The problem was then studied by C. A. Mills, who used the technic suggested by Vines. He reports that bleeding in hemophiliacs can be controlled at will by anaphylaxis. Mills studied four cases[2] and in three of these data concerning the treatment are given. In one case in which, apparently, the method of Vines was carried out, the clotting time was reduced from ten minutes to four and one-half minutes. In another case the clotting time was five minutes before a transfusion of blood from a patient sensitive to horse serum. Later 4 minims of horse serum were given and eighty minutes after this dose the clotting time had been reduced from ten minutes to two and one-half minutes. In the final case reported by Mills on September 25, the clotting time was five minutes. Then, forty-five minutes after an intracutaneous dose of horse serum (4 minims), the clotting time was three and one-half minutes. On October 4, it was four minutes, and one hour after the administration of serum (4 minims); the clotting time was reduced to two minutes and forty seconds. Commenting on this case Mills says: "We again see the corrective influence of the local protein reaction on the hemophiliac condition."

In these reports there seems to be lacking data necessary to establish any therapeutic value of anaphylaxis over a period of time sufficient to justify the procedure. The results of Mills are of the same nature as those of Vines in that the immediate effect of the serum injection was that of reducing the clotting time, but in no case is there evidence presented to show that during the period of reaction itself, was the clotting time influenced in any way.

Vines states that the duration of the effect of the treatment is dependent both on duration of the anaphylaxis and upon the severity of the hemophylic condition. In this respect it is possible that in the case here reported the clotting time was too high to expect a satisfactory reduction by means of the method advocated. However, Mills says: "This (procedure) is of very great value clinically, since we may now keep our hemophiliacs sensitized to some protein." And later he reports[3] "good success in five hemophiliacs by making them sensitive to sheep or hen serum," and that a study of their blood before and after the treatment shows that the fundamental fault has been corrected.

In order to test this hypothesis, it was decided to apply the procedure to a case of hemophilia, and observe the results over a sufficiently long period, to test its clinical value.

A. F. D., a white boy, aged twenty, entered the University Hospital on March 9, 1927, complaining of bleeding and painful, swollen joints.

The family history through four generations is charted. The patient's mother has been married twice. A half-brother of the patient died at five years from hemorrhage after having the frenulum linguae taken out by the family physician. One cousin also died at two years of age of hemorrhage.

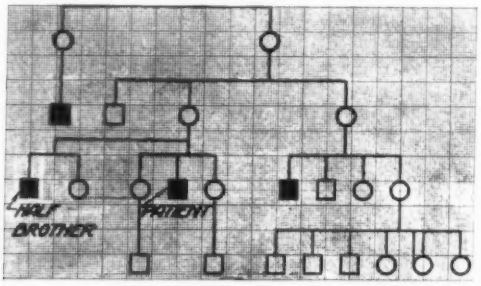

Fig. 1.—Family chart. Circles represent females. Black squares represent hemophiliacs. It will be seen that the Law of Nasse, "that the disease is transmitted by the unaffected female," is fulfilled and that the patient is a true hemophiliac.

At the age of six months the patient's mother observed dark spots on his body which were transient. At about five years of age he had swollen ankles with the overlying skin turning black and clearing; later, the knees and elbows became afflicted as well. Epistaxis was frequent in infancy but the joint symptoms were most prominent. At fifteen years of age he had hematuria over a period of three weeks. The bleeding episodes occurred at irregular intervals and during the past few years they apparently became less frequent.

The following data give a complete summary of the case through one year in chronologic order.

Admitted March 9, 1927. Bleeding time ten minutes; clotting time ninety minutes (Howell method). Urine clear. Hemoglobin 75 per cent; platelets 250,000. Blood type 4 (Jansky). The left leg was put in a cast in an attempt to correct a fixed equinus deformity of the left ankle.

March 25, correction being slowly obtained.

March 31, Cast removed and adhesive traction applied to obtain dorsiflexion of foot.

April 5, small hemorrhage into left ankle. Orthopedic treatment discontinued.

April 21, admitted to ward for nonspecific protein therapy.

April 22, patient given 500,000,000 killed typhoid bacilli intravenously at 4:30 P.M.

The white blood counts, during this experiment, varied between 7,900 and 16,600 quite parallel to the temperature curve. Tables I to III show the

TABLE I

TIME	W.B.C.	DIFFERENTIAL						CLOTTING TIME	TEMP.	PULSE	RESP.	PROTHROMBIN TIME	PROTHROMBIN QUOTIENT
		N	E	B	L	T	M						
Date 4/22/27													
4:00 P.M.	10060	59	5	0	23	1	12	60 min.	99.0	90	18	90 min.	
4:30 P.M.	500,000,000 typhoid bacilli injected intravenously												10.0
5:30 P.M.	7900	62	10	0	21	1	6	60 min.	100.0	78	16		
7:00 P.M.	9000	61	7	0	8	11	13	30 min.	102.6	120	14		
8:00 P.M.	7780	60	1	0	16	12	11	35 min.	102.0	108	14		
9:00 P.M.	13800	64	2	0	6	18	10	20 min.	101.4	102	14		
10:00 P.M.	12000	65	2	0	4	21	8	16 min.	100.4	108	12		
11:00 P.M.	16600	71	0	0	2	15	12	17 min.	100.0	102	10		
12:00 P.M.	15120	82	3	1	9	1	4	15 min.	100.2	96	12	85 min.	9.6
4/23/27													
1:00 A.M.	13460	72	1	0	14	7	6	15 min.	101.2	98	18		
2:00 A.M.	14400	65	1	0	32	57		15 min.	103.0	102	24		
3:00 A.M.	12600	60	2	0	28	2	9	18 min.	103.0	110	24		
4:00 A.M.	11040	86	3	0	7	1	3	20 min.	103.0	108	18		
5:00 A.M.	9400	77	1	0	20	1	1	20 min.	102.9	102	18		
6:00 A.M.	8920	81	3	0	12	2	2	20 min.	102.4	110	18	82 min.	9.1
7:00 A.M.	9140	63	2	0	29	2	4	20 min.	102.2	110	18		
5:00 P.M.	9820							50 min.	98.6	80	18		
4/24/27													
5:00 P.M.	9960							60 min.	98.6	80	20	85 min.	9.6

TABLE II

DATE	CLOTTING TIME	PROTHROMBIN TIME	PROTHROMBIN QUOTIENT
4/26/27	100 min.	85 min.	9.4
4/30/27	100 min.	56 min.	6.2
Pain in left leg and knee.	600,000,000 typhoid intravenously.		
5/ 3/27	95 min.	55 min.	6.1
Pain in right knee.			
5/ 8/27			
Right knee very sore.			
5/10/27	100 min.	60 min.	6.6
	700,000,000 typhoid intravenously.		
5/12/27	95 min.	53 min.	5.9
5/24/27	100 min.	60 min.	6.6
X-radiation over spleen.			
5/25/27	110 min.	65 min.	7.1
6/ 3/27			
X-radiation over spleen.			

TABLE III

HIGH PURINE DIET

DATE	CLOTTING TIME	PROTHROMBIN TIME	PROTHROMBIN QUOTIENT
6/ 7/27	102 min.	60 min.	6.6
6/22/27	95 min.	56 min.	6.1
6/27/27	97 min.	58 min.	6.4
6/29/27	95 min.	56 min.	6.1
7/ 2/27	24 min.	20 min.	2.2
7/ 7/27	107 min.	96 min.	10.6
7/11/27	95 min.	60 min.	6.6
Pain and swelling of left arm. Ice bag to elbow.			
7/13/27	100 min.	58 min.	6.4
Left arm very sore, unable to feed self.			
7/14/27			
Few R.B.C. in urine. Positive guaiac on stool.			
7/16/27			
Hemoglobin 80 %. R.B.C. 3,900,000. Eosinophils 1%.			
7/20/27	97 min.	60 min.	6.6
8/ 3/27	105 min.	90 min.	10.0
8/ 6/27	110 min.	85 min.	9.4
8/10/27 Patient discharged.			

TABLE IV

		CLOTTING TIME	PROTHROMBIN TIME	PROTHROMBIN QUOTIENT
12/ 9/27	- - - - - - - - - - - - - - - - -	101	74	8.2
12/10/27	Intradermal test with normal horse serum tried with negative results. 4.0 c.c. normal horse serum (P.D.) given subcutaneously.			
12/17/27	Wrists and hands swollen.			
12/19/27	Left arm swollen, painful at elbow - - -	72	54	6.0
12/20/27	0.2 c.c. normal horse serum (P.D.) injected intradermally. Marked reaction with development of large wheal and extensive hyperemia. Patient developed an urticaria 7 days after this injection.			
12/21/27	- - - - - - - - - - - - - - - - -	74	59	6.6
1/13/28	0.2 c.c. normal horse serum. Wheal and hyperemia. Blood in urine - - - - - - - - -	78	62	6.9
1/20/28	Effusion in left elbow joint.			
1/24/28	- - - - - - - - - - - - - - - - -	105	80	8.8
1/25/28	0.2 c.c. normal horse serum (P.D.) injected intradermally. Marked reaction with development of wheal and hyperemia.			
1/26/28	- - - - - - - - - - - - - - - - -	105	85	8.9
1/27/28	- - - - - - - - - - - - - - - - -	101	79	8.7
1/28/28	Blood in stool.			

changes which occurred. The data differ in no way from that usually obtained in normal individuals who receive intravenous protein except that the actual clotting time of the blood varied within wider limits.

This experiment occupied one month, the injections being given on April 23, April 30 and May 10. Between the second and third injections the patient developed a hemorrhage into the right knee associated with considerable swelling and pain. It seems clear, then, that the effect of shock therapy on this patient differs only in degree from that seen in experimental animals, and that the reduction of the clotting time is a temporary, rather than a permanent phenomenon.

The patient was discharged August 10, 1927, and he returned to the clinic December 8, 1927, for further observation with regard to the effect of anaphylaxis on the clotting time. Table IV gives in detail the results of the experiment. It is obvious that no favorable change occurred in the patient's condition while under observation. There were, on the contrary, continuous manifestations of hemophilia, such as hematuria and melena, as well as joint and muscle hemorrhages.

COMMENT

From the results in this case we are scarcely justified in assuming that anaphylaxis, or shock therapy, has a place in the treatment of hemophilia, and from the published results of Mills there is no indication that a lasting benefit was secured by maintaining an anaphylactic state toward foreign serum. Our results with protein shock therapy indicate that a considerable reduction in the clotting time may be secured *while fever is present.*

In considering the results of both Vines and Mills it must not be forgotten that, in many cases of hemophilia, there is considerable variation in the clotting time of the blood. For example in three cases reported by Davidson and McQuarrie[4] the variations were as follows:

CASE	LOW MINUTES	HIGH MINUTES
R. R.	33	70
E. H. T.	36	73
C. J.	70	387

In our own case the clotting time varied between twenty-four and one hundred and five minutes when the method of Howell[5] was employed. It will be noted in the tables that bleeding episodes were taking place at intervals regardless of the treatment employed.

SUMMARY

A case of hemophilia is reported in which an attempt was made to modify permanently the clotting time of the blood by anaphylaxis.

The method suggested by Vines (1921) was used but no permanent influence of the treatment could be detected in this case and the favorable results reported by Mills are not confirmed.

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THE EFFECT OF CALCIUM CHLORIDE UPON THE TOXICITY OF BILE*

By William C. Emerson, M.D., Detroit, Michigan

THE tremendous shock which follows the escape of bile into the peritoneal cavity has been repeatedly observed. Bunting and Brown[1] investigated this phenomenon by either injecting bile intraperitoneally or by dividing the gall bladder of rabbits and allowing the bile to escape into the peritoneal cavity. Death generally occurred in twenty-four hours and was attributed to direct action upon the myocardium.

A general bile pigmentation of increasing intensity may be seen in early obstructive jaundice clinically and there may be no accompanying symptoms. The picture is entirely unlike that seen in bile peritonitis. However, in each syndrome bile is the underlying etiologic factor. It would therefore seem that in obstructive jaundice the bile in the blood stream may be detoxified or there may be a depression of the liver function and all the normal elements of bile may not be escaping into the circulation. Mann and his coworkers[2] having shown the extra-hepatic formation of bile pigment, the degree of jaundice would not necessarily be an index of the actual amount of whole bile present in the blood stream and the tissues.

King and Stewart[3] investigated the effect of the injection of pig's bile upon the circulation of dogs and concluded that the toxicity was due to the pigment. They further observed that the blood calcium rose 20 per cent during the experiments. They believed that the rise in blood calcium was a protective mechanism in that calcium combined with the pigment and rendered it nontoxic. It was further concluded that the delayed coagulation time in obstructive jaundice was due to the calcium being combined, and there was none available for the process of coagulation.

Lee and Vincent[4] demonstrated the importance of calcium administration by mouth and intravenously to shorten the coagulation time of the blood in obstructive jaundice. Walters[5] has called attention to the fact that the high postoperative mortality rate in jaundiced patients was largely due to hemorrhage. He emphasized the importance of adequate preoperative preparation of such patients by the administration of calcium.

In view of the difference in the reaction of the organism to bile in obstructive jaundice and in bile peritonitis it seemed desirable to determine whether calcium had a true protective effect against toxicity. Were this demonstrated then the intravenous administration of a soluble calcium salt would be indicated as a preoperative measure in all cases of suspected bile peritonitis.

It would seem that if calcium were protective against the toxicity of bile

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Received for publication, July 12, 1928.

this might readily be demonstrated by determining the lethal dose of a given sample of bile injected at a uniform rate of speed and repeat the experiment but increase the available calcium by the simultaneous injection of a soluble calcium salt to determine whether the lethal dose was altered.

Meltzer and Salant[6] have shown that much of the confusion in the literature upon the relative toxicity of bile depended entirely upon the varied speed of injection used by different investigators. In all of our experiments we injected the bile at a uniform rate of speed, 2.5 to 3 c.c. per minute.

METHOD

Ox bile was obtained from twenty gall bladders of freshly killed animals. The material was diluted with an equal volume of normal saline and injected within three hours after its collection. Dogs were used in all the experiments and an effort was made to obtain animals of approximately the same weight to test a single sample of bile. The dogs were anesthetized by the open drop method and later a cannula was placed in the trachea and connected with an ether bottle. The femoral vein was exposed and a Hartman[7] two-way needle inserted. This was connected to two burettes containing the injection material. The diluted bile was placed in one, and the calcium chloride solution or normal saline in the other, depending upon the experiment. A 5 per cent solution of calcium chloride was used and the total amount to be given was injected simultaneously with the first 50 c.c. of bile. In the control animals an equal amount of normal saline was given. A cannula was placed in the carotid artery and connected with a mercury manometer for blood pressure tracings. A tube was placed in the etherization system and connected with a tambour

TABLE I

BILE SPECIMEN	WEIGHT OF CONTROL DOG POUNDS	LETHAL DOSE BILE PER POUND C.C.	WEIGHT OF DOG POUNDS	CALCIUM CHLORIDE PER POUND	LETHAL DOSE OF BILE PLUS CALCIUM CHLORIDE PER POUND C.C.
	pounds	c.c.	pounds	mg.	c.c.
5	18.0	10.2	24.25	10.4	9.0
1	18.75	10.5	23.25	10.9	7.6
4	21.5	6.8	23.0	10.9	5.8
2	19.75	9.7	21.25	11.9	8.7
2	19.75	9.7	16.25	16.0	8.6
2	19.75	9.7	15.25	16.6	8.6
3	11.75	6.9	14.5	18.0	7.2
1	18.75	10.5	13.0	19.0	9.2
3	11.75	6.9	8.5	29.4	8.1
7	15.75	8.8	16.75	30.0	6.3
6	12.5	8.4	16.5	30.3	8.3
6	12.5	8.4	7.5	33.3	8.0
5	18.0	10.2	13.75	36.3	6.3
7	15.75	8.8	13.0	38.4	8.5
5	18.0	10.2	12.5	40.0	7.2
7	15.75	8.8	11.25	44.4	6.0
5	18.0	10.2	10.0	50.0	6.5
7	15.75	8.8	10.0	50.0	5.4
6	12.5	8.4	9.75	51.3	8.2
Average		8.75			7.5

and writing lever for a tracing of the respiratory movements. Blood pressure and respiration tracings were made after the injection of every 25 c.c. of diluted bile.

RESULTS

The lethal dose of seven samples of bile was determined in control animals and the effect of the simultaneous introduction of calcium chloride in varying dosage from 10 to 52 mg. per pound of body weight was observed. It was not deemed advisable to inject greater quantities of calcium chloride because the usual therapeutic dose in man is 4 mg. per pound and according to Walters and Bowler[8] the lethal dose for a dog is 116.3 mg. per pound.

Fig. 1 shows the characteristic gradual fall of blood pressure which occurs when bile is injected into a systemic vein. There is a striking arrhythmia and at times an apparent cessation of cardiac activity.

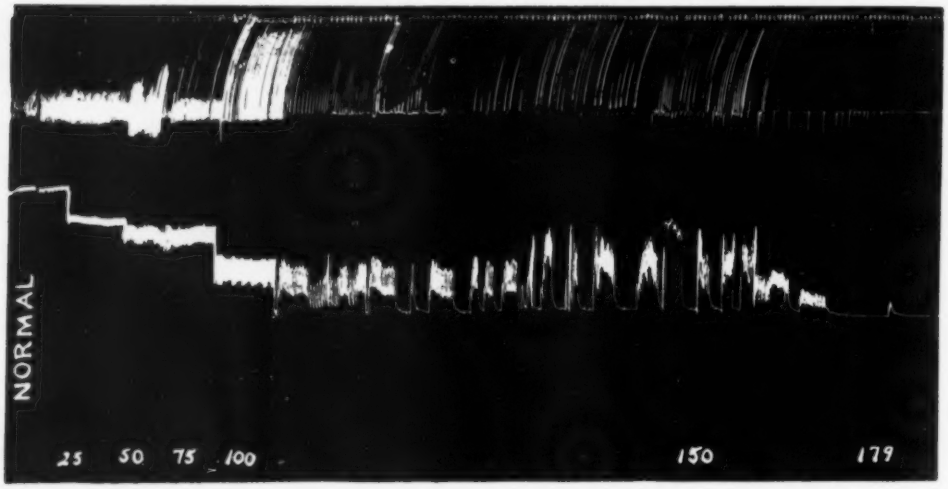

Fig. 1.—Effect on the blood pressure and respiration when bile is injected into the femoral vein.

Fig. 2 shows essentially the same picture. The dog weighed 13 pounds and was given 19 mg. of calcium chloride per pound. There is a similar fall of blood pressure and arrhythmia to that previously noted.

The results are summarized in Table I. The lethal dose in the control animals varied from 6.8 to 10.5 c.c. per pound while the average lethal dose for the group was 8.75 c.c. per pound.

The lethal dose of bile in 19 dogs which received calcium chloride varied from 5.4 to 9.2 c.c. per pound. The average lethal dose for this group was 7.5 c.c. per pound.

DISCUSSION

The experiments demonstrate that the simultaneous intravenous injection of calcium chloride in varying dosage with fresh ox bile definitely does not decrease the toxicity of the bile Although in both series of experiments the injections were made at a uniform rate, the lethal dose for those animals

which were given calcium chloride was somewhat less than in the controls. As the dosage of calcium was increased the difference was more striking. This is of interest because of the observations of Walters and Bowler[8] that the lethal dose of calcium chloride in a jaundiced dog was much greater than in a normal animal.

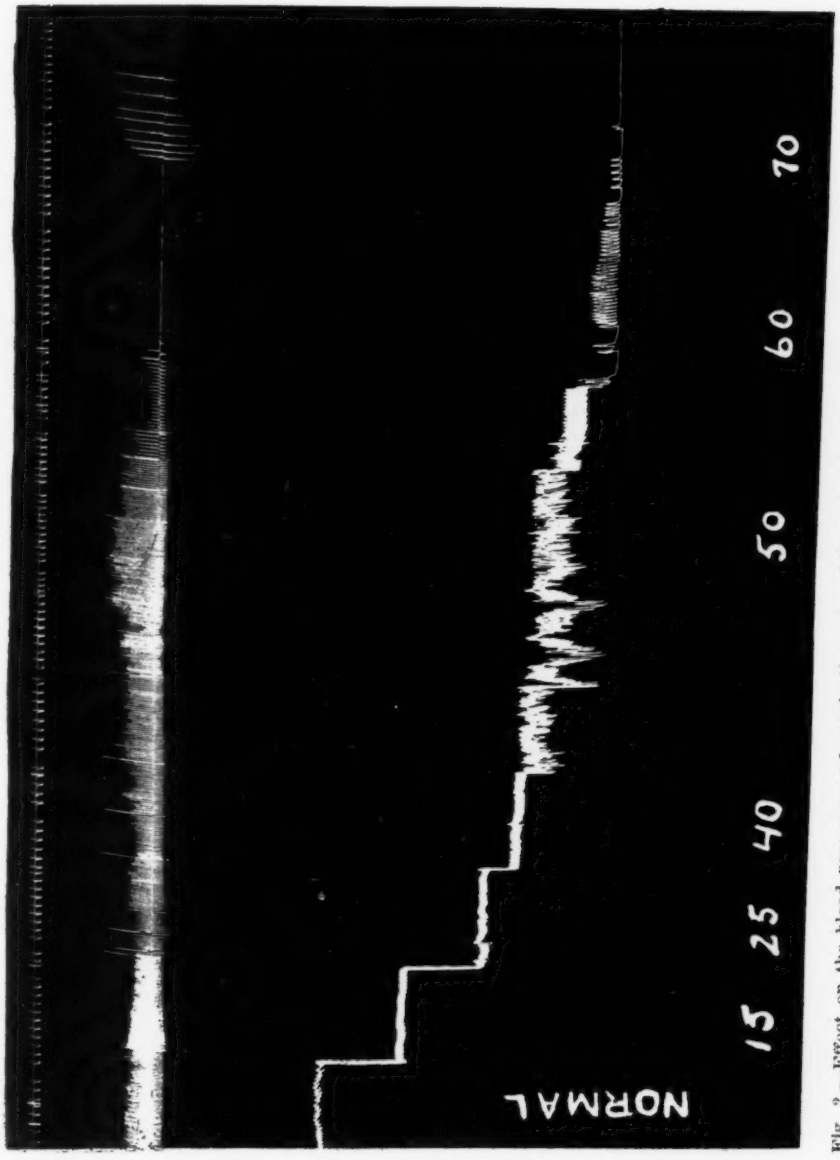

Fig. 2.—Effect on the blood pressure and respiration when bile and calcium chloride, 19 milligrams per pound, were injected into the femoral vein.

It would seem that the injection of bile resulted in the mobilization of calcium which enters the blood stream. The delayed coagulation time in obstructive jaundice may be due to the calcium being in a combined form and not being available for the process of blood clotting. However, the experi-

ments presented do not suggest that the calcium is combined with the toxic element or elements of the bile but rather to some other constituent, possibly the protein, and therefore is not protective.

CONCLUSIONS

1. Calcium chloride shows no protective effect against the toxicity of bile in the normal animal in doses varying from 10 to 52 mg. per pound of body weight.

2. The beneficial effect of calcium chloride in obstructive jaundice as preoperative preparation would seem to be limited to its effect upon the coagulation time of the blood rather than a true detoxifying action.

3. There is nothing to suggest that calcium chloride would be beneficial in fulminating bile peritonitis.

This work was suggested and done under the direction of Dr. Edward C. Davidson.

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THE ACTION OF COLD UPON THE T-WAVE OF THE ELECTROCARDIOGRAM*

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IN 1909 Eppinger and Rothberger[1] found that when the basal and right portion of the heart of the dog is chilled by the ethyl chloride spray the chilling exerts a positive influence upon the T-wave of the electrocardiogram. When it is applied to the apical and left portions of the heart, the opposite effect appears, and a negative influence upon the T-wave occurs. These observations upon the effect of cold upon the T-wave were confirmed by the work of Wilson and Herrmann,[2] and Smith,[3]† the former also pointing out

*From the Laboratory of Physiology, Faculty of Medicine, Paris.
Received for publication, August 20, 1928.
†Smith did not use ethyl chloride in his experiments because of Lewis' objection that it throws the muscle involved out of function.

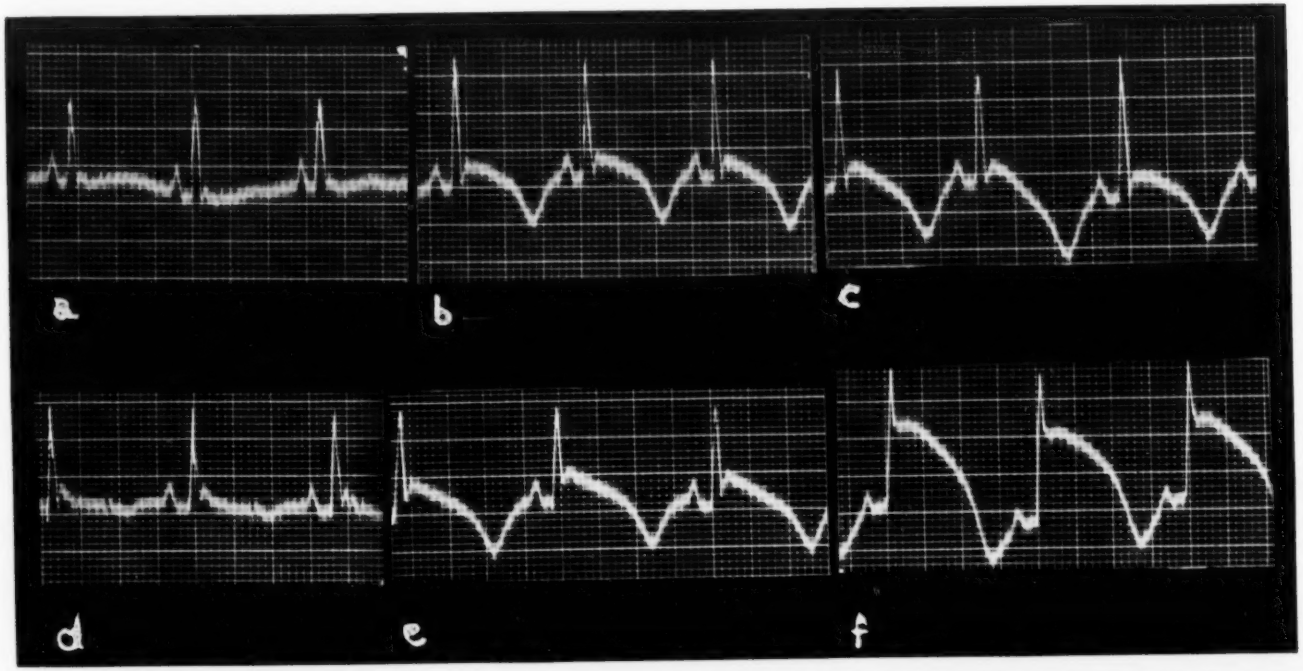

Fig. 1.—Electrocardiogram of the heart of a cat using axial (right arm—left leg) leading. Time in fiftieth seconds. *a*. The normal electrocardiogram. *b* and *c*. During the application of the ethyl chloride spray to the outer wall of the right ventricle. Great negativity of the T-wave ensues. *d*. After the curve has returned to the normal. *e* and *f*. During the application of the spray to the outer wall of the left ventricle. Note the negative action upon the T-wave which precedes the final effect.

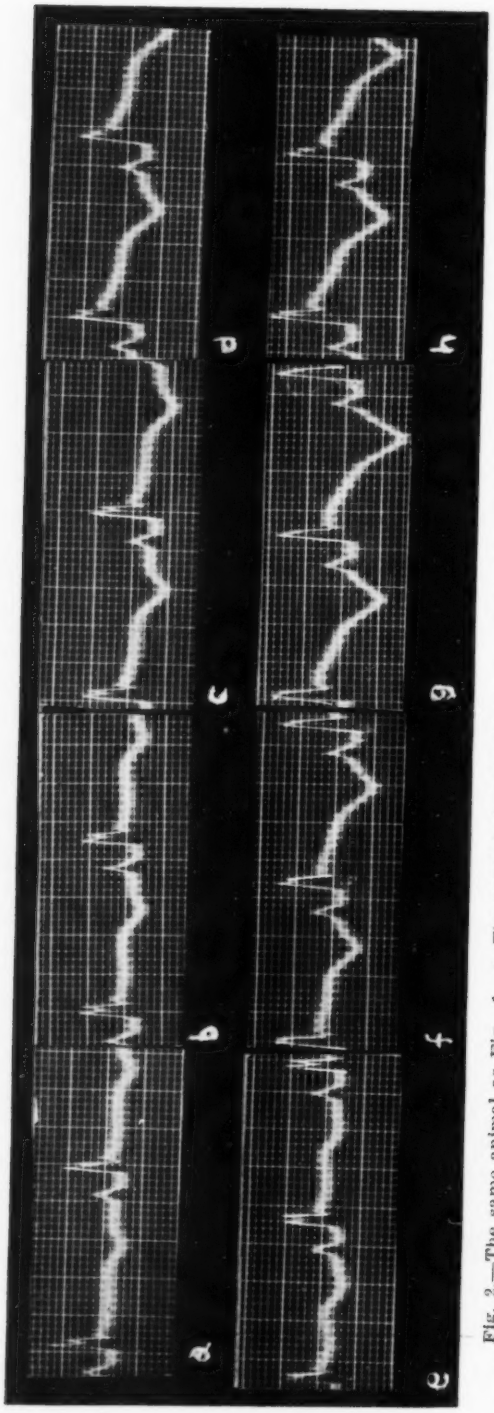

Fig. 2.—The same animal as Fig. 1. *a*. The normal electrocardiogram. Slight changes have occurred in its form undoubtedly due to the action of the ethyl chloride spray. *b*, *c* and *d*. During the application of ice to the outer wall of the right ventricle. Note that the effect is the reverse of the effect obtained with ethyl chloride. *e*. Ten minutes after the ice has been removed from the heart. *f*, *g*, and *h*. During the application of the ice to the outer wall of the left ventricle. Observe how the negative influence upon the T-wave in *g* passes into the reverse effect in *h* as the application is prolonged. Compare this with Fig. 1, *e*.

that since the effect of cold is to prolong the processes involved in muscle contraction, the T-wave changes induced agree with the assumption that it represents the decline of the active process in the muscle contraction. That this is the same for the human heart has been pointed out by Wilson and Finch.[4] These authors obtained cooling of the posteroinferior aspect of the apex of the left ventricle by having the subjects drink iced water.

When the ethyl chloride method is repeated upon the heart of the cat, these effects are exactly reversed, and the freezing of the basal right portion of the heart causes marked negativity of the T-wave, and freezing the apical left portion of the heart causes marked positivity of the T-wave. (Fig. 1.) If, however, the chilling of the heart is made less intense than that produced by the ethyl chloride spray, e.g., by the application of cold water or ice, the effects occur as these authors describe them and tend to reverse themselves as the action of the ice is prolonged (Fig. 2). Since the external ventricular walls in these areas of the cat's heart are sufficiently thin to be frozen by the ethyl chloride, there can be little doubt that the procedure throws the portion of the wall involved entirely out of function.

Although the effects in all the experiments* were not always pure the contrast of the results under the two sets of conditions, prolongation of and elimination from function, indicates that in the axial lead, actual right basal effects are associated with a positive influence upon the T-wave since prolongation of its activity, or lessening of left apical ventricular effects elevate the T-wave, and actual left apical effects are associated with a negative influence upon the T-wave, since prolongation of its activity or throwing muscle of the right ventricle out of function causes negativity of the T-wave. Furthermore, they suggest that when the summation of the events of the cardiac contraction are such as to favor the right ventricle by a prolongation of its activity (temporal preponderance) or an algebraic increase in the muscle activity over that of the left side of the heart (volume preponderance) the T-wave tends to positivity, and in the case of the left side of the heart, the reverse action or negativity of the T-wave is the result. There is, however, no sensitivity in this balance of the muscle activity of the right and left ventricles, inasmuch as a comparison of the effects of ethyl chloride in the heart of the dog and cat points out that the area involved must be large in its relation to the size of the heart before the appearance of an easily recognizable effect from a volume preponderance in the axial lead, since in the dog the spraying indubitably throws muscle out of function without the electrocardiogram showing the effect expected.

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*There were 10 trials in 5 cats under ether anesthesia, the order of investigation being ethyl chloride to right then left ventricles, then ice or ice water flowing through a fine tube to the right then left ventricles, with a sufficient interval between each step for recovery. The heart was always carefully protected with absorbent cotton so that only the area to be tested was exposed.

POSITIVE BLOOD CULTURES IN NONSPECIFIC ULCERATIVE COLITIS*

By Burrill B. Crohn, M.D., and Gregory Shwartzman, M.D., New York

THE subject of nonspecific ulcerative colitis has given rise in recent years to considerable discussion. It would seem that, as a clinical entity, this type of colitis is just assuming its real significance. The clinical symptomatology is not yet complete. New phenomena are still being described. Conceptions regarding the course and prognosis are in a state of continuous change. The treatment surely is in perpetual flux varying between absolute conservative medical management to extreme radical surgical methods. Probably the least determined point is the etiology and in this connection particularly the rôle that streptococci singly or in symbiosis play as a possible causative factor. It is particularly as bearing on the point of probable bacterial etiology of this disease that we offer this contribution dealing with cases of nonspecific ulcerative colitis in which positive blood cultures have been found. The recovery of positive blood cultures in this disease constitutes to the best of our knowledge an original observation.

Case 1.—A girl (E. S.) nineteen years of age, who two years previously had an attack of diarrhea with bloody stools. She had been severely ill for seven weeks under hospital treatment and was discharged improved. She remained well until five months before admission when she had a recurrence of her trouble; many daily movements containing blood, loss of appetite and of weight. Under intestinal irrigations and dieting, a certain amount of improvement took place. She was admitted, however, to Mount Sinai Hospital with the statement of having five to six bloody movements a day and of experiencing sharp, radiating pain with each bowel movement. She was pale, thin, undernourished; the abdomen was relaxed. There was marked tenderness over the course of the colon. The blood count showed a marked secondary anemia; hemoglobin 42 per cent, color index of 0.48 and a normal differential count. The stools were bloody and contained no parasites and no amebae. She had, however, a small perforation of the nasal septum and radiographically showed a mild clouding of the left ethmoid sinus.

By sigmoidoscopic examination, the mucous membrane was seen to be granular, hypertrophic, friable, bleeding easily and showing innumerable small pin point hemorrhages, but no definite ulcerations. The temperature on admission was 104° F; a febrile course at this level continued for several days but, in the course of two or three weeks, showed a gradual subsidence to almost normal. Two weeks after admission she suffered pain in the left lower extremity; on physical examination a bluish pallor of the left leg and toes was seen; tenderness was present along the left femoral vein. A tentative diagnosis of phlebitis and thrombosis of the left femoral vein was made. During the course of the next three or four days, the swelling of the left leg increased and then subsided without further physical signs.

Among the interesting clinical symptoms observed in this patient, there was a burning and painful sensation in the mouth and on the tongue. On examination one noted a moderately severe aphthous stomatitis, consisting of a small vesicular eruption scattered over the buccal mucosa and gingival surfaces of the tongue.

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Received for publication, July 27, 1928.

The course of the case was favorable; a constant and steady improvement taking place. One month after admission sigmoidoscopy still showed an advanced stage of severe colitis with innumerable ulcerations, pinhead to pencil-top in size. Acriflavin irrigation treatment was begun at this point. Some further swelling of the left leg took place without elevation of temperature. A definite diagnosis of femoral thrombosis could not be made though the symptoms were suggestive. The eruption on the oral mucosa continued for some time; the patient showing persistent evidence of small ulcers on the inferior surface and lateral margins of the tongue. The secondary anemia persisted; hemoglobin showing 51 per cent, and red blood count 3,400,000 cells. In the course of the next few weeks the patient made a steady improvment. Small doses of vaccine obtained from Dr. J. A. Bargen of the Mayo Clinic were given, accompanying the acriflavin irrigation treatments. The patient was finally discharged two months after admission very definitely improved.

Bacteriologic Data.—Several bacteriologic examinations of the stools were made and reported as follows:

July 10, bacillus coli and Streptococcus anhemolyticus.

July 15, enterococcus and bacillus coli.

July 18, bacillus coli communis and Streptococcus hemolyticus. Pus examination directly from the sigmoid mucosa showed enterococcus.

An anerobic blood culture taken on admission was negative. A routine aerobic blood culture taken eight days later, during the height of the febrile course, showed an anhemolytic streptococcus growing in the tomato glucose flasks.

CASE 2.—In brief, this is a history of a sixteen-year-old school girl (P. G.) who was admitted to the Mount Sinai Hospital complaining of a diarrhea of five months' duration. She had had from three to nine movements a day, the stools being loose, watery, and containing blood and mucus. She had lost ten pounds in weight; complained of weakness, increasing pallor and dry cough. Physical examination showed a small area of bronchopneumonia in the left interscapular region and a few râles at both pulmonary bases. There was a secondary anemia, hemoglobin being 30 per cent, red count 2,300,000, and white count 5,600, the only unusual characteristic being an eosinophilia of 6 per cent. The temperature on admission ranged between 104° and 105° F. A systolic murmur was present, at the apex of the heart, the murmur presumably being of hemic origin.

Sigmoidoscopic examination showed a markedly reddened mucosa with hemorrhagic infiltrations, but no definite ulcers. The membrane was friable and profuse bleeding followed the examination.

Within two or three days after admission it was noted that herpes was present on the lips. This herpes persisted for a few days and then gradually disappeared. The retinal field showed a few scattered petechial hemorrhages on several examinations.

The patient showed continuous improvement under treatment; the temperature subsided rapidly, but showed unaccountable irregular rises during the two months that the patient was in the hospital. Under acriflavin retention enemas, and forced general diet, the patient at the end of eight weeks was practically well and was discharged from the hospital.

Bacteriologic Data.—An aerobic culture was made upon this patient the day of admission at the time of the highest temperature (104° F.). A Streptococcus hemolyticus of the mucoid type was found in the fluid medium of the flasks. Subsequently, an anaerobic blood culture was reported negative. Agglutination tests of serum against strains of dysentery organisms were persistently negative.

In this case one positive culture of blood contained an hemolytic streptococcus. A mild bronchopneumonia was present at the time; there was, therefore, some doubt as to whether the colitis or the pneumonia was accountable for the blood stream invasion. Our experience has been that negative blood cultures are routinely obtained in ulcerative colitis as well as in mild cases of bronchopneumonia. The febrile reaction was quite evidently due to the severity of the colitis, because it continued irregularly for several weeks after the subsidence of the mild pneumonic signs and accompanied the fluctuations and severity of the colitis symptoms.

CASE 3.—(J. K.) A man of twenty-five years under observation on the wards of Mount Sinai Hospital for almost nine months. He had been suffering with symptoms of a severe colitis at irregular intervals during the preceding four years, during which time he had lost seventy pounds in weight. In May, 1927, an ileostomy had been performed with the hope of causing a reduction in temperature and improvement in the course of the disease. However, in October, five months after the operation, the patient was transferred to the medical wards for our care, the ileostomy having succeeded only in some slight general improvement. At this time sigmoidoscopy showed a severe generalized ulceration, with marked proliferative hyperplasia and polypoid regeneration. While under our observation he was treated with a vaccine furnished by Dr. J. A. Bargen of the Mayo Clinic. He received also gentian violet by mouth, irrigations with acriflavin and at other times with flumerin. Some weeks later we administered, intravenously, large doses of polyvalent antidysentery serum, which resulted in a slight immediate benefit, but no marked change in the course of the disease. On the basis that any benefit derived from vaccine or serum was in the nature of a nonspecific reaction, we gave the patient a series of typhoid vaccine injections, employing sufficient amounts to cause severe reactions.

The course of the disease was, however, slowly but progressively downward. Irregular temperature continued, symptoms of arthritis of the large joints supervened. At one time there was severe pain in the left perineum and in the tuberosity of the ischium, so that we suspected a periostitis. Radiographic studies were negative. Eventually, in desperation, a colectomy was suggested, the sigmoidoscopic picture giving one an impression that the damage to the mucosa was so extensive and the clinical course so hopeless that only a radical removal of the diseased tissues could offer any permanent hope. A colectomy was performed which resulted fatally five days after operation, due to the marasmic condition of the patient and a postoperative infection of the abdominal wall.

On various occasions cultures obtained from the wall of the intestines through the sigmoidoscope showed bacillus coli, bacillus pyocyaneus, and on two occasions an enterococcus. One blood culture taken in November was negative, but the two blood cultures taken later at the peak of a rise in temperature showed enterococci in the fluid culture media. Another blood culture taken four months after these findings was negative.

TECHNIC OF BLOOD CULTURE EXAMINATIONS

The methods employed for cultivation of blood were, as follows:

Twenty-one c.c. of the patient's blood were inoculated at the bedside into flasks each containing 100 c.c. of fluid medium and into tubes each of which contained 19 c.c. of agar. The fluid media consisted of one flask each of 2 per cent glucose broth, plain broth, and tomato broth, each of which received 5 c.c. of blood. In addition, one tube each of plain agar, 2 per cent glucose agar and liver-hormone agar were melted, cooled to 45°, and inoculated with 2 c.c. of blood and poured into Petri dishes. Four c.c. of ascitic fluid were added to the liver-hormone agar at the time of the addition of the patient's blood. The inoculated media were then subcultured each on a series of various media daily for four to five days and spreads were made.

The tomato-broth flask employed in this laboratory consisted of 90 c.c. of 2 per cent glucose broth and 10 c.c. of sterile tomato extract. The tomato extract was made by boiling ripe tomatoes. The fluid extracted in this manner was centrifuged, filtered through Berkfeld V candle and adjusted to P_H 8.2 before use. In many instances the tomato-broth was found extremely useful for blood cultures, since tomato extract serves as a source of powerful growth-promoting factors (Thjötta and Avery,[1] Shwartzman[2]).

DISCUSSION

It should be pointed out that under the above described conditions the positive results obtained in a very large number of patients suffering from various diseases could be divided into two groups:

A. *Positive findings in all media.* Under this group of positive blood cultures bacterial growth was obtained in fluid media in abundance and in addition in solid media in the form of isolated colonies. Such results were obtained in all frank bacteremias. On no occasion was there growth in solid media without growth in fluid media.

B. *Positive findings in fluid media.* Frequently, bacteria grew in all fluid media or in some of them, while the solid media remained sterile for the entire period of observation. It was felt that in such cases the initial number of organisms present in the blood was extremely small. In fact, while 5 c.c. of blood cultivated in enriched fluid gave rise to a culture, the reduction of blood from 5 c.c. to 2 c.c. and its cultivation in solid media, in which there was some mortality of initially seeded organisms, resulted in sterile cultures. There was a large group of cases which gave positive findings in fluid media alone (mostly tomato and glucose flasks alone).

The positive findings in fluid media alone were obtained in conditions which could not be termed septicemias. They were in the nature of transient bacteremias. Septic foci communicating freely with the general circulation could not be detected clinically nor were such conditions found at postmortem examination. In many of the cases there were no clinical signs which would point to the presence of bacteria in the general circulation. Frequently, a rise of temperature for a day or two with subsequent speedy recovery was the only symptom. Positive blood cultures in fluid media were also obtained on several occasions in acute rheumatic fever, debilitating conditions such as malignant tumors, tuberculosis, acute anterior poliomyelitis and other similar conditions. The organisms found in this group of cases were mainly streptococci, mostly nonhemolytic and sometimes green producing streptococci.

In the cases of ulcerative colitis reported above positive blood cultures in fluid media were obtained. The organisms found in the 3 cases were Streptococcus hemolyticus, Streptococcus nonhemolyticus, and enterococcus.

A nonhemolytic streptococcus was termed an enterococcus if the organism grew in 2 per cent bile broth, resisted heating to 60° for twenty minutes and fermented esculin.

It remains to determine the significance of the positive blood cultures in the above reported cases of nonspecific ulcerative colitis.

There is always first the possibility of direct absorption of bacteria into the general circulation from the upper respiratory and intestinal tracts. Bull and McKee[3] recently reported experiments on rabbits in which they were able to induce bacteremia by depositing organisms on the surface of the nasal mucous membrane.

Libman pointed out on several occasions that streptococci are common secondary invaders of the blood.

Desoubry and Porcher[4] established the fact that bacteria of many sorts may pass through the intestinal mucosa during the digestion of fatty substances and are found for a few hours in the chyle and in the blood. As pointed out by Calmette,[5] the findings have been verified so often that it is now a rule in all institutes of serotherapy to bleed the horses only when fasting, if it is desired to obtain sterile sera.

The interpretation of the relation of the positive blood culture to the etiology of the disease must be made with great caution. It should be remembered that the initial number of organisms found in the cultures of the blood was very small. The organisms recovered were of the type which was commonly found in transient bacteremias. On the other hand, no definite basis can be offered for denying that these organisms may be responsible for the etiology of the disease itself.

CONCLUSIONS

1. Positive blood cultures can be obtained under certain conditions in cases of nonspecific ulcerative colitis.

2. Possible interpretations of such findings are developed but it is recognized that considerable experimental and clinical work is necessary before definite conclusions can be drawn.

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PROGRESS IN HAY FEVER*

A Review of the Hay Fever Literature of the Years 1927 and 1928

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IT HAS been my feeling that there is need for a review of the recent work on hay fever, a review so nearly complete as to be of value to the allergist who has not the proper access to many periodic publications, and to the internist, otolaryngologist, and others interested in this field of work and who wish to keep themselves abreast of the times with a minimum of time and effort. In this article are abstracted the periodic publications of 1927 and 1928. There has been an earnest attempt to include all the articles available in the original in English, as well as German, French, Italian, and other foreign journals. However, there were three or four articles which the reviewer has been unable to obtain. I wish to point out the fact also, that in reviewing any subject one is faced with the difficulty of abstracting articles which because of the detailed

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or tabular nature of their contents do not lend themselves easily to condensation. These articles are included in this review, but the reader is strongly urged to read them in the original if he is at all interested in the suggested contents.

Duke[1, 2] has reviewed the advances in our knowledge of allergy in the last two years. However, because of the breadth of the scope of his papers, he has been unable to put much stress on the subject of hay fever. Although nothing radically new has occurred in this field in the last two years, it is gratifying to note nevertheless that considerable progress has accumulated in our knowledge of the etiology and treatment of this disease.

ETIOLOGY AND PATHOLOGY

Although many reports appear in the literature concerning the causes of hay fever as a phenomenon of sensitization to pollen, most of these are repetitions of facts already recorded in the past.[3] The all-important question of the basic mechanism underlying the predisposition to sensitization has not yet been answered. In the meanwhile, however, there have appeared several articles which throw considerable light on heredity and other factors in the etiology.

Clarke et al,[4] in a study of heredity in allergy, show that not only is the allergic tendency inherited, but also the organs or tissues involved are more or less predetermined by heredity. They present statistics showing that pure asthmatic subjects have more pure asthmatic antecedents than pure hay fever antecedents; and that pure hay fever subjects have more pure hay fever than asthmatic antecedents.

Balyeat,[5] in a study of the factors governing the acquisition of hypersensitiveness, concludes that inheritance is the principal factor determining whether or not an individual will develop clinical hay fever or asthma, and chiefly governs the time of life when symptoms appear. He also maintains that heredity influences the kind of atopen to which the individual will become sensitive. The earlier in life sensitiveness occurs the greater the likelihood of becoming sensitive to more than one group of atopens. Degree of exposure to a given substance is also a deciding factor whether sensitiveness will develop to that substance. Balyeat has demonstrated also that a child may be born specifically sensitive to a food protein, sensitization occurring probably by placental transmission of atopens.

In a series of 36 individuals who had an hereditary background of hay fever Baldwin[6] has attempted to determine the relation of the onset of the cutaneous sensitivity to the clinical symptoms. Of this number five developed positive skin tests simultaneously with the development of hay fever; in a sixth case of hay fever the skin tests did not become positive until the second season.

Bristow[7] says that "It (allergy) seems to be a disease of civilization, hay fever among negroes is rare." This is not at all in accord with the experiences of the reviewer who sees many cases of hay fever and asthma among negroes in the Clinic. This is substantiated further by recent reports of considerable

hay fever in South Africa, and asthma in India. It would seem that in those countries in which hay fever is rare, it is more a matter of botanic than racial considerations.

Vaughan[8] makes the observation that Weil maintains that the difference in quantitative distribution of smooth muscle among different experimental animals accounts for their different shock organs: rabbit, pulmonary arterioles; guinea pig, bronchi; dog, liver; etc. This is offered as a possible explanation by Vaughan as to why some allergics develop hay fever, others asthma, and still others cutaneous manifestations. He also considers the following etiologic factors of importance, especially from the standpoint of treatment; the conception that pollinosis is a constitutional disease; that specific local sensitization may play some rôle; that factors deciding nasal localization may be nonspecific, such as infection and mechanical obstruction; and that coexistent sensitization to food or other inhalants may aggravate or localize the hay fever.

Balyeat[9] emphasizes the importance of orris root in allergy. In 373 cases of hay fever he has found it to be a factor in 7.2 per cent. He says that the majority of these orris-sensitive individuals must be desensitized.

Duke[10] has found that in hay fever cases 10 per cent had nasal polyps and 6 per cent sinus infection. He considers this secondary to allergy rather than primary, and warns against nasal operations in such cases. In a study of 91 patients who had a nasal complaint in addition to their presenting symptoms, Eyerman[11] found that in the hypersensitive cases 72 per cent showed eosinophiles in the nasal secretions, while in the nonallergic cases only 9 per cent showed occasional eosinophiles.

Grafton Tyler Brown[12] made an analysis of the eosinophile counts in asthma, hay fever, and hyperesthetic rhinitis. His conclusions are that in asthma the average eosinophilia is as high in allergic as in the nonallergic cases, and that in hay fever and hyperesthetic rhinitis the blood eosinophilia is equally high and presents no difference in the sensitive and nonsensitive cases. As a result of a study of eosinophilia at various stages of hay fever Sternberg[13] suggests that the increase of eosinophiles in hay fever seems to be a shock reaction. He calls attention to the fact that no increase was observed in patients free from symptoms before and during their treatment.

In a series of 85 cases studied, comprising 53 cases of asthma, 20 of hay fever, and 12 nonseasonal allergic rhinitis, Sterling[14] finds a hypocalcemia in only 23, while phosphorus deficiency (0 to 3.0 mg. per 100 c.c.) was present in 68.

As in the past one still finds in the literature allusion to fanciful, unsubstantiated hypotheses attempting to explain the pathology and treatment of hay fever. Thus, Leach[15] claims that all sensitization, hay fever, asthma, etc., is a result of nasal pathology. He maintains that the nose acts as a filter to prevent the entrance of toxic substances into the blood, that when nasal pathology is present filtration is not adequate, hence asthma or hay fever. Byrd[16] states that hay fever is due to pathologic currents (electrical?) which may enter the nose via the nasal ganglia or nasal nerves. This author tries to

explain almost every disease by the conception of pathologic currents in which the brain is the distributing center and abnormal conditions result from "shorts" of excessive currents.

CLINICAL MANIFESTATIONS AND DIAGNOSIS

According to Balyeat[43] the general health of the hay fever patient is better than the average and he has a greater resistance to infectious disease. This is true in spite of the fact that in the autumnal hay fever cases 28 per cent had asthma during the season and 60 per cent tend to develop asthma eventually.

Kahn[17] states that in chronic hay fever or asthma in the South there may be in addition to the allergic symptoms a chronic toxemia due to pollen. This occurs mainly in children and consists principally of backward growth, backward mentality, undernutrition, sallow skin, and enuresis.

Pollen hay fever and asthma in children does not seem to have the adult picture, according to Kahn[18, 19]; it is usually mild and is frequently mistaken for symptoms of adenotonsillar hypertrophy. He says that a definite bronchitis, accompanying the rhinitis, precedes the initial asthma. At that stage the allergic nature of the manifestations may be recognized by coexisting vasomotor rhinitis, pollen precautions, and relief with epinephrin. High fever and leucocytosis often accompany the asthmatic attacks and may lead to a diagnosis of pneumonia. To this the reviewer would like to add that similar errors arise in asthma in children due to things other than pollen. Many of the acute respiratory conditions in children diagnosed as pneumonia, tonsillitis, or infectious bronchitis are either asthma or allergic bronchitis. Other essential differences in pollinosis of children and adults as noted by Kahn are: the mildness of the nasal symptoms in children, the definite toxemia frequently occurring, and the less marked diagnostic skin tests.

Lamson,[20] in a series of intracutaneous tests with pollens in over 100 hay fever cases shows a distinct specificity as to species and genus. Many of the papers, such as that of Hurwitz,[21] corroborate further the diagnostic value of skin tests. Kahn[22] again reiterated his previous statements in regard to the unreliability of skin tests. He claims that negative skin tests, both cutaneous and intracutaneous, are so frequent in the presence of pollinosis as to make such tests undependable. He advocates the use of subcutaneous tests: daily increasing injections subcutaneously until a reaction is obtained. He admits, however, that he may have to push the injections to as high a concentration as 1:20 before reactions occur. He states[23] that negative skin tests (with as high as 2 per cent pollen intradermally) are frequent in Texas in pollen asthmatics in infants and young children especially. He adds, however, that the longer the duration of asthma the less likelihood is there of negative skin tests. It may be said that Kahn's experiences are not in accord with those of the reviewer nor with those of the majority of allergists. However, peculiar climatic conditions in the South may possibly account for this difference.

In a report of further observations on the nonspecific diagnosis of allergy I[24] have presented a series of cases of asthma, hay fever and perennial rhinitis in whom a diagnosis of allergy could be made by the intradermal test with

human dander extract. The results in hay fever, however, were not as good as in asthma: in 38 cases 36 reacted with the dander extract. Although this reaction is of no great practical value in hay fever, it is of great value in perennial rhinitis or asthma.

EXPERIMENTS WITH ATOPENS AND ATOPIC REAGINS

Lamson, Piness, and Miller[25, 26, 27] in a series of experiments with spring and fall hay fever cases, showed that there is a definite diminution of skin reactivity after pollen treatment. They say that skin desensitization to fall pollens results in a majority of cases after the usual course of treatment. This persists at least for four weeks, and in some cases for months or years. In a study of a number of allergic patients in whom negative reactions were obtained to certain pollens the injection of such pollens did not sensitize the skin. They also failed to engender skin reaction to certain pollens by injecting them into a person definitely hypersensitive to other pollens. From this they rightly suggest that natural exposure, inhalation of pollens, or injection of the same pollens, are not the only factors necessary to induce skin hypersensitiveness in an allergic person.

On a large number of pollen-sensitive patients Huber[125] made a study of the skin reactions to dry large and small ragweed pollen grown in different sections of the United States. The samples of the same species of pollen varied considerably in color due probably to the method used in collecting and preserving them. With the same species of pollen some patients consistently gave greater reactions to one or more of the samples than to the others, while other patients gave greater reactions to different samples. Huber interprets this variation in reaction to mean that the chemical composition of the samples varied and that all patients were not sensitive to the same constituent.

The disputed question of the identity of the atopens of the short and giant ragweed has been studied further by Aaron Brown.[28] The latter maintains that these atopens are entirely identical, and he supports his claims by the following observations. In a series of 31 late hay fever subjects tested with extracts of both giant and short ragweed they were found to be equally sensitive to both. One group was given a series of prophylactic treatments with one extract and the other with the other with results as good as in combined treatment. After a maximal tolerance had been established for one extract an equivalent or larger quantity of the other extract was injected without being followed in any case by a constitutional reaction.

In experiments on rabbits the Scandinavian authors, Thomsen and Baage[29, 30] have found that pollen injections caused a high antibody titer. This was quite specific for the species but showed quantitative relationship to members of the genus of pollens. In such highly immunized animals they found no evidence of sensitization as could be determined by intracutaneous or subcutaneous injections or conjunctival application. On the other hand, Loeb[31] was able to sensitize guinea pigs with watery extracts or alcohol precipitates of pollen (Pinus silvestris) and goose feathers.

Grove[32] attempted to produce local hypersensitiveness in lower monkeys and chimpanzees by the method of passive transfer originally described by

Prausnitz and Küster and found to be highly successful in experiments on man. In this work the pooled serum containing the atopic reagins for ragweed, horse serum, La Page's glue, and cotton-seed were used. Her conclusions are that the results are practically negative as regards the production of local or constitutional hypersensitiveness.

Black[33] cites several reasons for throwing doubt upon the relation of the atopic reagin to the true atopen in pollen. He says that negative skin reactions may occur in clinically sensitive cases and positive reactions may occur in absence of clinical sensitiveness. In the reviewer's experience this certainly occurs very rarely in this part of the country (Chicago district). Again, he says, skin reactions may continue unchanged in individuals successfully treated, and they may persist long after spontaneous cure of the patient. The size and intensity of the skin reaction have no relation to the severity of the clinical condition. Finally, he claims that in mixtures of reagin and atopen the reagin was neutralized while the atopen remained unchanged. As a whole his arguments are well-founded and should stimulate further experiments in this field.

REGIONAL BOTANIC REPORTS

In the last two years there has been considerable progress in the accumulation of botanic surveys of hay fever plants in various parts of the United States and in some foreign countries. This demonstrates, more than any other fact perhaps, the general appreciation of the importance of the hay fever problem and its relation to the local flora.

G. T. Brown[34] reports on the late spring or summer hay fever in the District of Columbia and vicinity. He incriminates the following: sweet vernal grass, June grass, orchard grass, timothy, red top grass, and English plantain. Bernton[35] records 3 cases of hay fever due to the pollen of the paper mulberry tree (Papyrius papyrifera Kuntze) in Washington, D. C. He says that this tree, although a native of Eastern Asia, grows in the eastern half of the United States from New York to Florida.

In the Colorado Springs district of Colorado Conway[36] reports the most important hay fever plants to be Russian thistle, lamb's quarter, sage, ragweed, and cottonwood tree. In addition to his complete and classic botanic survey reported on in 1926 Waring[37] reports seven cases in which the cotton of the cottonwood tree was probably a cause of "hay fever." Tests with pollens of the usual plants pollinating at that time of the year were negative, while the intradermal injection of the cotton extract was positive. All of these patients were also sensitive to the pollen of the cottonwood.

In Oregon, Chamberlain[38] considers 95 per cent of the hay fever to be of the midsummer type and caused by the grasses. The other plants of importance are Russian thistle and the artemesiae. From pollen counts and a botanic survey of the east shore of San Francisco Bay, Rowe[39] concludes that in that region the grasses pollinate all year round, but are most profuse from March to August. The trees pollinate from January to July. Ambrosiae, artemesiae, and chenopodiae are causes of hay fever, but are not very profuse. Of the ambrosiae, the Western ragweed (ambrosia psilotachya) is the one found in that region.

Although it is now accepted opinion among the vast majority of allergists that brightly colored plants and flowers are not a cause of hay fever, Stewart,[40] writing from Iowa, seems to consider also the rose, dandelion, goldenrod, goldenglow, daisy, aster, dahlia and others as contributing factors. In western Montana, Foss[41] reports three hay fever seasons: spring (March to April) due to the trees, early summer (May and June) due to grasses, and late summer (July to frost) due to Russian thistle, sagebrushes and poverty weeds. The last group, according to the author, accounts for 60 per cent of all hay fever cases in that region. In Nevada a somewhat similar situation is reported by Albert[42] on the basis of skin tests made on 31 hay fever cases. He says that the Russian thistle, pollinating from July to October, is the most important hay fever plant; other chenopods, shad scale, annual salt bush, and lamb's quarter are also important. As a group he finds the grasses more important than the chenopods, and the amaranths of least importance.

Balyeat[43] surveys the botanical situation in Oklahoma and concludes that the important hay fever plants are: Bermuda grass (May 15 to frost), amaranths (pigweeds), Western water hemp, and the ragweeds. In the middle southern states Spitz[44] reports the three reasons as they are known in the more northern states with the important additions, perhaps, of Bermuda and Johnson grass in the former.

A survey of the Salt River Valley in Arizona by Phillips[45] shows the false ragweeds, franseria acanthicarpa, f. tenuifolia, and f. delthoidea, to be of great importance. The Western ragweed (ambrosia psilotachya) is apparently only a local cause of hay fever. Bermuda grass is important in that region, but he especially stresses the importance of the false ragweeds. He says that patients who are sensitive to ragweed but have never been exposed to false ragweed react clinically to the latter almost immediately. Sometimes, however, it may take as long as three years.

Balyeat[46] finds that the Western water hemp (acnida tamariscina) is a very important factor as a cause of hay fever and asthma in Oklahoma. As a result of data collected from replies to questionnaires sent to professors of botany of colleges of several states he reports that there is a great prevalence of acnida tamariscina in Oklahoma, eastern and northern parts of Texas, eastern Nebraska, southern part of South Dakota, Iowa, and southern Michigan. There is a lesser prevalence of this plant in Illinois, Missouri, Arkansas, and Kentucky; there is a small amount in Ohio and New York. From these reports also it is shown that the closely related plant acnida tuberculata is prominent in Iowa, Wisconsin, Minnesota, and southern Michigan. Balyeat states that in Oklahoma he finds the Western water hemp responsible as a sole cause in 3.6 per cent, and as a definite contributing cause in 35 per cent of the hay fever cases.

Scheppegrell[47] made inquiry from public officials of Mexico, and various sections of Central and South America in regard to the prevalence of hay fever. The Mexican government and several states in South America reported that there is practically no hay fever there, while several states in Central America absolutely denied the existence of hay fever in their localities. It was Scheppegrell's opinion that hay fever in most of these countries is prob-

ably uncommon and also little understood. In Argentina Van Boeckel[48] reports that the early summer type of hay fever exists there and is apparently due to the grasses. They have also apparently a tree season.

There are several rather meager reports concerning the botany of hay fever in European countries. Hansen[49] of Heidelberg finds grasses to be the predominating cause in Germany. Von Bánszky[50] thinks that there is not much less hay fever in Germany than in America. The German hay fever season occurs chiefly during May and June and is due principally to timothy, but also to other grasses, such as orchard grass, red fescue, June grass, etc.; and to grains, rye, wheat, and oats. The early spring and fall types are due to flowers, he thinks. In Denmark hay fever was studied by Baage[51, 52, 53] with local pollens. Although the number of his cases is small he has been able to arrive at rather definite conclusions. He found that all of his cases reacted to the six grasses, timothy, orchard grass, June grass, wild oat, ray grass, and meadow foxtail. All reacted to corn pollen. There were no reactions to wheat, oat, or barley pollen. The tree pollens gave only weak and occasional reactions. He thinks that some of the fall cases are due to the artemesiae. The most important hay fever season is from the first of June to the middle of July. Dekker[62] reports two cases of hay fever in winter due to the Alpine grass pollen in the mattress. Both cases showed positive skin tests and were relieved by the removal of the mattresses.

In a preliminary communication Pirie[54] reports on the hay fever situation in South Africa. The grasses seem to be the most important factor, but none of the timothy family (genus phleum) is present. He considers the compositae also important because they are so numerous that close contact cannot be avoided. A rather peculiar cause of hay fever in that part of the world is the pollen of the pepper tree (Schimus molle). This tree is insect-pollinated, but under special conditions such as a dry high wind, and because of the great numbers of these trees used for street and road decorations in many localities it may cause considerable hay fever. From the same region Jurriaanse[55] reports two cases of hay fever treated with mixed extracts of the pollen of compositae, artemesiae, and pepper tree, with good results.

POLLEN STUDIES AND COUNTS

Studies in morphology and counts of atmospheric pollen have taken great strides in the last two years. Durham,[56] with the aid of data obtained from several allergists has reported comparative ragweed pollen records for nine large cities. It is interesting to note what enormous differences exist in the pollen concentration of the air of these cities. Oklahoma City, with a maximum count of 3600 ragweed pollen per cubic yard, is at the top of the list. Next in order are Indianapolis, Kansas City, and Chicago, the latter having a maximum of 250 (in 1925). New Orleans and New York are next in order, the latter having a maximum of 100. The cities having the lowest counts are Philadelphia; Richmond, 12; and Oakland, 6.

Rowe[39] has made the first report in which pollen counts were recorded for an entire year. On the east shore of San Francisco Bay he shows that pollen is present in the air practically every day throughout the year, except on

the days it rains continuously. In Rowe's articles are to be found drawings of the California pollen and key tables for their morphologic identification.

Duke and Durham[57] made comparison of variation of pollen content of the air from day to day in different localities in Kansas City, Missouri. They conclude that the variation of the severity in the patient's symptoms are explainable entirely by the variation in pollen count. Comparison of these pollen counts with those made in other cities, and those in other seasons, also offers an explanation to these authors why a given method of treatment may be successful in one city, or one year, and be almost a total failure in another city or in the same city in another season.

The factors which determine the pollen content of the air are analyzed by Balyeat.[58] He considers, of course, the abundance of plant life as the most important factor in determining the amount of pollen. Wind causes greater pollen counts and increases hay fever symptoms. Greatest pollination occurs during the morning hours. Rain frees the air of pollen, but subsequent sunshine may entirely overcome that factor. Before pollination rain helps weeds to grow larger and to produce more pollen.

Duke[59] determined the pollen content of air under various conditions of stillness. In a room kept closed for one and one-half years no pollen was found in the air. In a room closed for one day there were 3 pollen per cubic yard; in a room closed for one day with a fan going for twelve hours, 8 pollen; and in ventilated rooms on the same day, 20 pollen. The outside air count at the same time was 191. Experiments near the propeller of an aeroplane show pollen counts to be 100 or more than of still outside air. From these observations the author concludes that still rooms can be utilized for pollen-sensitive patients on bad days or nights, and that motor rides, train trips, and aviation should be avoided.

Identification of pollen by structural features are discussed at length by Piness and McMinn.[60] They give a complete key to pollen identification of California pollen, but they emphasize the following points. Most grasses have pollen grains resembling grains of half-ripe field corn. In the compositae group the artemesiae pollen have elliptical form with furrows: the ambrosiaciae are oval with definite spicules. All wind-borne spiculated pollens belonged to the ambrosiaciae. The chenopodaciae (goosefoot family) have spherical pollen with round surface concavities. An abstract cannot possibly do justice to this article, and in order to study this subject the reader is urged to see the original publication, in which there are many microphotographs, tables, etc. In a very interesting but technical paper Wodehouse[61] attempts to demonstrate how a study of the structural character of pollen grains may be utilized in the identification of plants and their classification and phylogenesis. In this article he discusses chiefly the ambrosiaciae pollen which he says can be recognized by their tricolpate character, short spines, and short furrows.

CHEMISTRY OF POLLEN

Whether or not the active principle of pollen is protein in nature is still a disputed question. Melli[63] of the University of Padua claims to have demonstrated that the active portion of pollen is protein, is undialyzable, becomes

inactivated by the action of proteolytic ferments, is destroyed by precipitating it with dealbuminizing substances, and provokes a positive Abderhalden reaction. These conclusions were arrived at by digestion experiments with pepsin, erypsin, trypsin, enterokinase, and papain.

Attempts to separate the individual proteins of pollen have been made by Bernton, Jones and Csonka.[64] From timothy pollen they have isolated four active proteins, which they have designated proteose A, proteose B, albumin, and glutelin. Of 19 grass-pollen cases 63 per cent showed cutaneous sensitiveness to two or more proteins, 21 per cent to proteose A, and 15 per cent to albumin only. The albumin constitutes one-ninth of the total extractable protein content. The albumin-sensitive patients have failed to derive benefit by treatment with extract of whole pollen, but were relieved by treatment with albumin fractions. Untreated albumin-sensitive patients had no asthma proteose-sensitive patients showed 72 per cent incidence of asthma. These workers made similar analyses of ragweed pollen and obtained proteose and albumin fractions. In 50 per cent of these there was skin sensitiveness to proteose and albumin; in 35 per cent to proteose alone, and in 14 per cent to albumin alone.

Alles and Lamson[65] have attempted to obtain purified pollen extracts by washing the pollen with 80 per cent alcohol and then following immediately by glycerol-saline extraction. This removed a considerable amount of the inactive substances, but the resulting extract was not quite as strong as the unpurified extracts. However, in three individuals this purified extract gave a strong reaction whereas they were negative to the crude extract.

Stier and Hollister[66] find that Russian thistle pollen extracts deteriorate rapidly. They found that the best extracting medium was 7 per cent sodium chloride with 46 per cent glycerin. They say that the glycerinized extracts are the only extracts of Russian thistle pollen which will retain their potency for a number of months.

Working with the pollen of orchard grass Loeb[67] precipitated an albumin fraction with 90 per cent alcohol. This precipitate was tested on 7 hay fever patients and found to give a good cutaneous reaction but somewhat weaker than the aqueous extract of whole pollen. The alcohol-filtrate gave no reaction. Some of the alcohol-precipitate was digested with 5 per cent trypsin and dialyzed. This digested alcohol-precipitate gave no reaction in the hay fever patients. Loeb concludes that the active principle of pollen extract is an albumin.

SPECIFIC TREATMENT

That the results of specific therapy continue to be good is evidenced by the contents of almost every paper on hay fever. Wynn,[68] in an excellent historical review, describes the evolution of the modern method of treatment. Others, such as Chamberlain,[38] report small series of cases which exemplify the benefits conferred upon the hay fever sufferer by pollen treatment.

There are many reports analyzing the results of treatment by specific methods. All are more or less in agreement that the majority of pollen cases can be benefited. Thus, Piness[69] says that in more than 90 per cent of

his cases there has been some degree of relief. Bernton[70] reports improvement in 94 per cent of his cases. In an analysis of 980 patients observed during the season of 1926 Vander Veer, Cooke, and Spain[71] find that 85 per cent received from 75 per cent to 100 per cent relief. These authors confirm the observations of other allergists that the pollen asthmas have a greater percentage of complete relief (75 per cent) than the pollen coryzas.

Conway,[36] reporting on 30 cases of hay fever treated at Colorado Springs, finds that all were relieved and 27 were entirely free from symptoms. Foss[41] of Montana, in a series of 184 cases over a period of four years, obtained relief in about 90 per cent. Those obtaining complete relief, however, constituted only 10 per cent.

In the foreign literature one finds increasing enthusiasm in the results of specific therapy in hay fever. I have already referred to reports from Argentina, South Africa, Denmark, etc. Freeman[72] of England writes favorably of pollen desensitization. In Germany Von Bánszky[50] and Strebel[73] report satisfactory results with pollen treatment, the latter author obtaining complete relief in 21 out of 40 cases. Loeb and Petow[74] comment upon the fact that specific desensitization is used very little in Germany. In 29 patients treated in various ways, with mixed grass pollens, single grass pollens, and peptone, the best results were obtained with mixed grass pollens. Hansen[49] reports 85 per cent of patients improved in a series of 23 cases treated by him. From Denmark comes additional endorsement of the efficacy of specific therapy in an article by Schwartz.[75] On the other hand, Hanhart of Zurich,[76] basing his experience on 3 cases of hay fever, reports discouraging results with pollen extracts.

Causes of Failure.—While the results of pollen therapy as a whole are good there are many cases in which there is complete failure; and this may constitute the majority in the hands of men who have had little experience with this form of therapy. I[77] have called attention to the fact that although with present methods of treatment one cannot produce relief in one hundred per cent of sufferers, nevertheless, attention to certain factors will lead to maximum results. These factors are: the early preseasonal treatment continued into the season; the individualization of dosages of pollen and intervals of treatment according to the patient's reaction; the careful determination of the species of pollen to be used; and finally, the preservation of the potency of the pollen extract by refrigeration in concentrated form.

Cohen, Reicher, and Breitbart[78] express similar views. They say that causes of failure in hay fever treatment are due to incorrect diagnosis; inadequate or improper treatment, due either to impotent extracts or insufficient number of injections or inadequate final dosage; the inability to develop tolerance; and contact with an unusually large dose of pollen.

Curability.—Very little has been said in the past about the curability of hay fever. However, as time passes and the number of cases treated thoroughly and repeatedly for many seasons is accumulating, we are coming to the realization that it is possible for hay fever to be permanently relieved. Thus, Henry[79] says that successful desensitization for 3 to 5 seasons frequently gives permanent immunity. Walker[80] analyzes a series of 100 "ap-

parently cured'' hay fever cases. He defines the word ''cure'' as freedom from symptoms for two or more years without treatment. Of this number 78 have gone three or more years without treatment. These patients were treated at weekly intervals for a period of thirteen to sixteen weeks previous to the pollen season. In 22 cases the pollen tests have become negative; in 22 they were very doubtful when treatment was discontinued; in 12 cases there was marked change in the reaction and in 44 no marked change occurred. Walker's tables seem to show that the average duration of treatment in these cured cases is three and one-half seasons, with a minimum of 1 and a maximum of 6, and that after two seasons the patient is usually free from symptoms (in those that go on to ultimate cure). He concludes that when pollen tests become negative it seems to be safe to stop treatment; in those in whom tests do not become negative it is desirable that the patient should have at least one season, and preferably two, of complete freedom from hay fever while being treated before treatment is discontinued.

Constitutional Reactions.—The greatest barrier, perhaps, to the acceptance of specific pollen therapy by the general medical profession and the public has been the risk of constitutional reactions. Vander Veer et al[71] find that in a series of 980 cases treated 10 per cent had constitutional reactions at some time during their course of treatment, while these reactions occurred in one per cent of all injections. They also found that these reactions are more apt to occur toward the end of a course of treatment. They blame overdosage as the most important factor and also mention two other causes, accidental injection into a venule and the change from an old to a new extract during the course of treatment. Bernton[81] states that constitutional reactions are due to overdosage of pollen extract or accidental intravenous injection. To avoid the latter he withdraws the needle partially after insertion and changes the axis slightly. To avoid serious reactions he advises two precautions: to have adrenalin at hand, and to have the patient wait for fifteen to twenty minutes after pollen injections. To the above the reviewer would like to add that intramuscular injections which are frequently given with intent of subcutaneous administration, and intracutaneous injections, further increase the possibility of constitutional reactions.

Kahn[18] calls attention to the fact that dosages and methods of administration of pollen extracts in children do not differ from those in adults.

The controversy of the relation of the two important ragweed pollens, short and giant ragweed, has been opened again. Aaron Brown[28] finds that treatment with one pollen protects against both pollens as well as treatment with both. On the other hand, treatment with ragweed pollen did not protect in the slightest degree against grass pollen[82] as indicated by natural exposure during the season or reaction to subcutaneous injection. He concludes that only one variety of ragweed pollen is necessary for the treatment of ragweed cases, but that the grass pollen must be used for grass cases.

Perennial Treatment.—One of the most promising advances in specific therapy is the development of a method of treatment whereby the patient is treated all year round. This plan of treatment, as described by Aaron Brown[83, 84] consists of the injection of pollen at three- to four-week intervals,

instead of the usual preseasonal short interval method. However, this should only be tried after the individual has been worked up to a large dose by the usual method. In addition to the fact that possibly the therapeutic results may be better Brown cites the advantages that treatment may be started at any time of the year and the actual number of treatments may be lessened. Kahn[85] has independently described a somewhat similar plan. He states that to maintain immunity to pollen he gives a maximum dose every week, sometimes as long an interval as three weeks, and given indefinitely. He says that this method can be modified by holding the protection by a weekly dose of one-tenth or one-fourth of the maximum protective dose and running up the treatment a few weeks before the onset of the following season. Vander Veer et al[71] also describe the perennial method of treatment which they say they have used since 1920, but have used it on a larger scale since 1925. They give monthly injections of the maximum dosage attained during the season. They claim that their results are fully as satisfactory as by the preseasonal method and that it aims at permanent immunity.

Oral Treatment.—That pollen extracts may produce immunity when given orally has been demonstrated by Black.[86, 87] His method consists of giving orally, three times daily, a 5 per cent extract of pollen in a glass of water or milk. The initial dose is 10 drops and every dose is increased by 10 drops until 60-drop doses are taken. The administration is begun with the first appearance of hay fever symptoms. In a comparison of 73 orally treated with 61 hypodermically treated patients he finds that oral administration has many advantages although protection is not secured as frequently. Strebel[73] combines injections with oral administration. The latter consists of giving four times daily 50 drops of his special pollen extract "Pollysatin" in a half glass of water.

Pollen-Free Atmosphere.—The benefit derived from residence in pollen-free resorts is too well-established to allow of any dispute. Cohen[88, 89, 90] has continued his observations in obtaining such a pollen-free atmosphere at home. By means of a motor-driven suction fan which sucks in air through a window opening and filters it through layers of felt a pollen-free room can be obtained. In such a room a pollen-sensitive patient may remain free from symptoms, or be completely relieved of his symptoms in two to seven days' residence if his manifestations are well established. To keep a patient comfortable the use of such a pollen-free atmosphere from eight to twenty-two out of every twenty-four hours will be required, depending on the individual's tolerance.

EPINEPHRIN, EPHEDRIN, AND SIMILAR DRUGS

Balyeat[91] finds epinephrin useful in simultaneous injection of pollen extracts in cases which have a tendency to systemic reactions. He admonishes, however, that in such cases adrenalin must be continued in subsequent injections of the pollen.

Ephedrin, the alkaloid obtained from the Chinese plant Ma Huang, has continued to demonstrate its sphere of usefulness in hay fever as well as in asthma. There are several papers dealing specifically with the use and results of ephedrin therapy; there are also many other papers dealing with other fea-

tures of hay fever or asthma in which the use of ephedrin is alluded to. It has been found possible to obtain results in either of two ways: locally, by spraying or applying solutions to the mucous membranes; and systemically, by oral administration.

Wilkinson[92] reports the use of ephedrin orally in 5 cases of hay fever with good results. Leopold and Miller[93] obtained relief in 5 out of 11 cases treated. Althausen and Schumacher,[94] reporting the results in 62 cases of hay fever and asthma, claim benefit in 80 per cent of their cases. Gaarde and Maytum[95, 96] describe the results obtained on 36 cases of autumnal hay fever treated with ephedrin, orally, locally, or combined. Of this series only 11 had had preseasonal pollen treatment. Ephedrin orally (25 mg.) gave almost complete relief, lasting four or more hours, in 54 per cent of the cases, and partial relief in 16 per cent. In 10 cases of pollen asthma 5 were relieved and 3 only moderately relieved. The use of a 3 per cent ephedrin spray two or three times daily resulted in marked relief in 28 per cent of their cases, and only fair results in 48 per cent. No relief was obtained in the asthma cases by the latter method. Piness and Miller[97] obtained relief in only one case out of 5 treated orally, and 18 out of 20 treated locally. Green[98] reports good results from local application of ephedrin in 2 cases of hay fever. He tried it in only one case by the oral method with no relief.

Balyeat[99] suggests a new use for ephedrin, its employment prior to injection of pollen extract to prevent constitutional reactions. For this purpose he advises the patient to take 35 mg. ephedrin orally fifteen minutes before the injection of pollen, or 50 mg. when the reaction begins.

The use of ephedrin is limited considerably by its frequent production of undesirable symptoms. The most constant effects, as mentioned by most authors,[93, 94, 96, 97, 99] are palpitation, tremor, nervousness, insomnia and nausea. There are also described by others such effects as weakness, vomiting, perspiration, and a sense of constriction around the head. Althausen and Schumacher[94] have obtained undesirable effects in 30 per cent of their cases, and in 12 per cent so severe that the therapy had to be discontinued. Gaarde and Maytum[96] found such marked toxic effects in 16 per cent of their cases as to make its use in those instances impossible. Piness and Miller[97] report untoward results in at least 20 per cent of their cases. Balyeat[99] states that in 20 per cent of his cases (hay fever and asthma) the symptoms were so severe that the drug was discontinued.

From the above observations and from my own experience with ephedrin therapy I am of the opinion that: (1) ephedrin has some field of usefulness as an adjunct in the treatment of hay fever; (2) the results are more evident in the cases that are treated by preseasonal pollen injections and may be negligible in untreated cases; (3) its usefulness is greatly limited by its tendency to produce undesirable symptoms; (4) locally, it has some beneficial, but rather short-lived effects; (5) it has in no way changed the status of, nor the necessity for, desensitization therapy.

Because of the undesirable symptoms and because of its rather high cost, attempts have been made to synthetize drugs similar to ephedrin. Kreitmar[100] reports experimental findings with the use of a new drug which he calls

"ephetonin." Effects on blood pressure of normal and decerebrated cats, on the heart, respiration, bronchial musculature, and virgin uterus of guinea pig were the same with ephetonin as with ephedrin. Fischer[101] says that ephetonin is chemically identical with ephedrin, except that the former is optically inactive. He states further that the pharmacologic action is the same, but it has the advantage in that it is cheaper. Berger and Ebster[102] obtained remarkable results by spraying the nose and eyes of hay fever cases with 5 per cent ephetonin. Their conclusion with respect to the oral use of ephetonin (in asthma) are: that its action is slower than ephedrin, that equal doses produce less effects, and that there are cases benefited by ephedrin and not by ephetonin. They claim the following advantages for ephetonin: that undesirable effects are much less, that it is cheaper, and that it may be used as substitution therapy in cases of tolerance to adrenalin or ephedrin.

Miller and Piness[103] report a new synthetic substitute for ephedrin. It is the sulphate of phenolethanolamine. Orally it has only a slight effect on hay fever or asthma, but locally, in 2 per cent solution, it has as good an effect as 3 per cent ephedrin.

OTHER MEDICINAL TREATMENT

A number of reports deal enthusiastically with the use of various drugs in hay fever. Sterling[14] recommends the use of calcium and phosphorus, especially the latter, and states "since our administration of phosphorus in addition to desensitization in hay fever patients the results have been almost 100 per cent freedom from symptoms."

Nussbaum[104] of Germany used "phocajod," an iodine preparation, on himself and several other cases with good results. Hamburger[105] recommends the bromides very highly. He says that one gram of sodium bromide mornings and evenings and one-half hour prior to going out doors will keep most cases free from symptoms. He says further that the effect may last a few hours to two or three days. Nitrohydrochloric acid is strongly recommended by Beckmann,[106, 107] basing his experience on 17 cases of hay fever, including himself. He claims to have obtained 100 per cent relief in all of these patients by the administration of about 10 minims of nitrohydrochloric acid in two-thirds of a glass of water after each meal and upon retiring.

Some of foreign literature contains mention of modes of therapy which to the American observer seem very strange and fantastic. Thus, Bonjour[108] recommends morphine in small doses, basing his contentions on his use of it in the past thirty-five years. Proby,[109] among other remedies, speaks of the use of a hydro-alcoholic ether extract of jaborandi. Billard[110] recommends the mineral waters of Mont-Dore on the supposition that hay fever is due to a change in the P_H concentration of the nasal reactions. His explanation is as follows: Each pollen and each female flower have their own P_H reaction. Fecundation can only result when the pollen of certain P_H reaction come in contact with the ovum. If the pollen of the wrong P_H concentration reaches the ovum no fecundation takes place. He regards the contact of pollen with the mucous membranes of the eye, nose, etc., as "monstrous fecundation." Whether or not clinical symptoms will result in a particular individual de-

pends on the P_H reaction of his nasal secretions. Usually a too high P_H causes a breaking up of pollen and a toxic reaction. He, therefore, claims to have obtained very remarkable results with the use of mineral waters in hay fever.

Hirsch and Loewe[111] describe a new drug, the lactone of gallic acid-ethanolaminochlorhydrate, which they call "salvamin." This drug is chemically allied to adrenalin, is water- and alcohol-soluble, occurs in colorless crystals, and has about one-hundredth the toxicity of adrenalin. They claim to have produced remarkable results in 50 hay fever cases by the use of this drug in doses of two to six tablets of three-fourths grains daily. Failure occurred in only two instances. These patients were able to go in fields, take auto rides, train rides, etc. Hirsch[112] further says that in the administration of this drug to 1000 individuals he has found it absolutely nontoxic. In another communication[113] he stresses the fact that no undesirable effects are produced by this drug, and again relates his experience with the 50 hay fever cases previously reported on. He says, however, that these cases were treated during the 1925 and 1926 seasons, which, he admits, were very mild seasons. Another report on this drug comes from Gerlach[114] who cites the good effects produced on himself, a hay fever sufferer for eighteen years.

Spiess[115] thinks that hay fever is a disturbance of the balance of internal secretions. On the basis of the fact that dermatologists had obtained excellent results with thymus extract in psoriasis and other skin lesions and that many of asthmatics had had a previous skin disturbance, it occurred to him that thymus treatment might be of value. He cites his success in several cases of asthma and hay fever. In hay fever he begins therapy with the onset of the first sneezing and gives one to two ampoules of "thymophorin" intramuscularly daily for 10 to 15 doses. He also suggests the use of calcium in addition, and also stimulation radiation of the thymus.

NONSPECIFIC AND LOCAL THERAPY

The French school seems to be fond of nonspecific methods of treatment of hay fever. Thus, Vallery-Radot and Giroud[116] employ intradermal injections of 50 per cent solution of de Witte's peptone. They begin on the second or third day of the symptoms and give daily injections for twenty days, and notice results mostly on the last injection. The dose is 0.1 c.c. with daily increments of 0.1 c.c. Flandin[117] believes better results are obtained with intradermal autoserotherapy, and after fourteen years' experience with this method he is convinced that that is the best mode of treating hay fever patients.

Duke,[10] Hurwitz,[118] and others agree that nasal surgery must be done with distinct fear in a case of hay fever or asthma. Nevertheless, according to Hurwitz, and observations by others, there are times when nasal infections may be a predisposing or aggravating cause of hay fever. In such proper cases it may be considered advisable to do some local surgery.

Leach[15] recommends some kind of local treatment which he calls "filtration method." Just what the nature of his treatment is seems quite in doubt from the contents of the article. Hollender and Cottle,[119] in discussing the treatment of hay fever and asthma, advise the substitution of morphine for adrenalin, recommend the use of ultra-violet therapy by general body expo-

sure and intranasal application, use calcium and thyroid orally, and employ nasal tamponage for one-half hour with argyrol. Kagan[120] speaks of "detoxication," relieving body and nose abnormalities, Dowling tamponage, ultraviolet exposures, x-ray treatment (for asthma), but admits that "a further treatment when indicated is pollen extracts as a preventative and curative measure.

Hamm[121] cites the cure of hay fever in 20 cases by diathermy with special nasal applicators. A. and L. Pokorny[122] cite two cases of hay fever in physicians who obtained remarkable results with x-ray therapy directed to the root of the nose.

While practically all of the above nonspecific methods of treating the hay fever patient are unproved, uncorroborated and unconvincing, there are some encouraging results reported by American workers with vaccine therapy. These men are careful and experienced hay fever specialists, modest in their claims and cautious in their assertions. Thus, Grafton Tyler Brown[34] says that "cold" vaccines are a valuable adjunct to pollen therapy in coseasonal treatment. Scheppegrell and Thiberge[123] analyze the effects of vaccine therapy in a series of 1399 hay fever patients. They used vaccines only during the season and gave it every other day. They conclude that vaccines used in this manner increase the percentage of good results. Three types of vaccines were employed: (1) the usual mixed catarrhal-respiratory vaccine, used in cases in which the acute attack seems to be due to infection; (2) vaccine consisting of organisms based on bacterial tests of a large number of hay fever cases during acute attacks, and employed in cases in which symptoms appeared to be due to increased pollen infestation; (3) and autogenous vaccines, used in cases which did not respond to stock vaccines.

As in reports on therapy in other fields, some of the literature on hay fever therapy is rather confusing from the standpoint of its evaluation because each writer reports his own results by his own particular method without great attempt at personal comparison with other methods. Especially in specific pollen therapy there are many variations in technic, preseasonal and coseasonal, early preseasonal, intensive method, long-interval method, large and small maximum doses, adjuncts such as vaccine therapy, light therapy, etc. Ramirez,[124] in the most illuminating clinical paper in the last two years, has attempted to crystallize all of these questions in one report. He made a statistical analysis of 425 cases of hay fever treated by him from 1919 to 1927 and classified them into various classes, groups, subgroups, series, etc., according to variations in methods of treatment. Each question is answered by the results of treatment in an individual series of cases. From these studies the author draws the following conclusions: "It is evident that the best results are obtained in cases coming about three months before the expected date of pollination, receiving two weeks intensive treatment, increasing the dose of pollen rapidly and continuing injections up to the pollen season, reaching as high a dose as possible, then continuing injections of pollen twice a week throughout the entire season; in addition, injections of autogenous vaccines prepared from the nose, and local treatment to the nose and eyes with increasing concentrations of pollen solutions, preseasonal and seasonal."

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LABORATORY METHODS

A SUGGESTED MODIFICATION OF THE KRAMER-TISDALL METHOD FOR THE MICROCHEMICAL ESTIMATION OF IONIZABLE CALCIUM IN BLOOD PLASMA*

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IN VIEW of the wide use which is being made of the Kramer-Tisdall[1] method for the determination of calcium in blood, it has seemed appropriate to publish the results of a study, which has been made in these laboratories, of the original method and of later modifications by Tisdall[2] and by Clark and Collip.[3]

Many attempts at recovery of calcium from a solution of known concentration using the Tisdall technic, modified to the extent of one additional washing of the calcium oxalate precipitate, yielded irregular values.

At first it was suspected that the low results obtained were due to the slight solubility of calcium oxalate in the 0.5 per cent NH_4OH (2 c.c. of NH_4OH sp. gr. 0.90 diluted to 100 c.c.) used in washing. Although this error was obviated by using 0.5 per cent NH_4OH previously saturated with calcium oxalate the results obtained were very irregular, and still below the known value of the solution.

Our next observation was that calcium oxalate precipitated from a solution of pure calcium chloride, although well packed after centrifugation was not matted as was the case with calcium oxalate precipitated from blood plasma.

Subsequent experiments using the modified Tisdall technic demonstrated that added calcium could be quantitatively recovered from blood plasma, notwithstanding the fact that its recovery from calcium solutions was uncertain. It is very probable that small amounts of fibrinogen and other protein are absorbed by the calcium oxalate, and are thus responsible for the more adherent precipitate obtained from blood plasma, rendering loss on decantation less likely.

The best results at recovery of calcium from a known aqueous solution of calcium chloride were obtained when the original technic of Kramer-Tisdall was followed, with the slight modification that 0.5 per cent ammonium hydrate saturated with calcium oxalate was used in washing. From the data in Table I, it is also evident that three washings are necessary to remove excess oxalate.

If the precaution is taken of using a centrifuge tube of not more than 3 to 4 mm. in diameter at the tip, the fluid supernatant to the calcium oxalate

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Received for publication, July 19, 1928.

TABLE I

DOG PLASMA

NUMBER OF TIMES WASHED	NUMBER OF DETERMINATIONS	MEAN VALUE MG. PER 100 C.C. OF PLASMA	MEAN VARIATION MG. PER 100 C.C. OF PLASMA	KNOWN CALCIUM VALUE; MG. PER 100 C.C.
1	4	10.66	±0.10	10.00
2	4	10.25	±0.05	10.00
3	3	10.13	±0.03	10.00

TABLE II

RABBIT PLASMA

SERIES	NUMBER OF TIMES WASHED	NUMBER OF DETERMINATIONS	MEAN VALUE MG. PER 100 C.C.	MEAN VARIATION; MG. PER 100 C.C.	SEPARATION OF WASH LIQUID
1	3	5	13.60	±0.10	decantation
2	3	3	13.55	±0.05	siphoned off
3	3	5	18.65	±0.15	decantation

precipitated from serum or plasma may be removed by decantation (Tisdall's technic) equally as well as by the siphon method used by Kramer-Tisdall as is shown in Table II.

In Series 3 of Table II, 5 mg. of calcium were added, and almost complete recovery realized.

From Table III it is apparent that the solubility of calcium oxalate in dilute ammonia water is not a serious factor unless it is allowed to remain in contact with the precipitate for some time. Our proposed modification of using ammonium hydrate previously saturated with calcium oxalate obviates any chance of loss by solubility, and renders the method more convenient, as the precipitated calcium oxalate may be left for any length of time in contact with the wash liquid.

TABLE III

DOG PLASMA

METHOD OF WASHING PRECIPITATE	NUMBER OF DETERMINATIONS	MEAN VALUE; MG. PER 100 C.C. OF PLASMA	MEAN VARIATION; MG. PER 100 C.C. OF PLASMA	WASH AMMONIA REMOVED BY DECANTATION
0.5% ammonia water	3	9.20	±0.05	at once
0.5% ammonia water saturated with calcium oxalate	4	9.20	±0.10	at once
0.5% ammonia water	3	8.90	±0.10	24 hr. later
0.5% ammonia water saturated with calcium oxalate	3	9.25	±0.10	24 hr. later

The modified Kramer-Tisdall method as herein described, when applied to plasma, separated from blood rendered noncoagulable by heparin, does not give the total calcium present, but only that which is in sufficiently ionizable form to be precipitated as the oxalate. In order to determine the total calcium in serum, plasma, or whole blood it is necessary to destroy the organic constituents, and then analyze the ash for calcium.

REAGENTS

1. Heparin (Hynson, Westcott and Dunning).

2. Four per cent Ammonium Oxalate Solution. Dissolve 40 gm. of pure ammonium oxalate in water and dilute to one liter.

3. Saturated Solution of Calcium Oxalate in 0.5 per cent NH_4OH. Precipitate some calcium oxalate from a calcium chloride solution with an excess of ammonium oxalate. Then wash by decantation and centrifuging until the washings are free from excess oxalate and NH_4Cl. The filtered off precipitate is then transferred to the 0.5 per cent NH_4OH solution (prepared by diluting 2 c.c. of NH_4OH, sp. gr. 0.90 to 100 c.c.) and shaken for one hour in a shaker. It is next allowed to settle, and the supernatant liquid filtered through a quantitative filter paper. The residual calcium oxalate may be used with a fresh lot of 0.5 per cent NH_4OH solution.

4. Approximately $N.H_2SO_4$. Dilute 28 c.c. of concentrated H_2SO_4 (sp. gr. 1.84) to one liter.

5. Standard N/100 Potassium Permanganate Solution. Dissolve 0.316 gm. of pure potassium permanganate in redistilled water and dilute to one liter. After standing for several days standardize against sodium oxalate.

For the standardization proceed as follows: Dry sodium oxalate of the highest purity in an oven at 100 to 105° C. for ten to twelve hours. Weigh off accurately, exactly 0.67 gm. thereof and dissolve in redistilled water, add 5 c.c. of concentrated H_2SO_4 and dilute to one liter. This is a N/100 sodium oxalate solution. By means of a burette measure off 25 c.c. portions thereof into 100 c.c. Erlenmeyer flasks, add 1 c.c. of concentrated H_2SO_4 to each, warm to about 70° C. and titrate while hot with the $KMnO_4$ solution. Determine the correction factor on the $KMnO_4$ solution. It is necessary to restandardize the $KMnO_4$ frequently,* but the $Na_2C_2O_4$ solution with the H_2SO_4 present is said to keep indefinitely. One c.c. of the N/100 solution is equivalent to 0.2 mg. of calcium.

Procedure: To 2 c.c. of serum or heparinized plasma in a 15 c.c. centrifuge tube, add 2 c.c. of distilled water. Mix well and then add 1 c.c. of the 4 per cent ammonium oxalate.

Again mix well and set aside for one hour. The centrifuge tube should have as fine a tip as possible; 3 to 4 mm. in diameter at the tip is a satisfactory size. After standing for one hour the tube is covered with a rubber cap and centrifuged for ten minutes at high speed, about 2300 revolutions per minute. The supernatant solution is removed either by decantation and finally inverting the tube for a moment upon filter paper, or by siphoning the supernatant fluid out by applying suction to a pipette drawn out to a very fine point. The latter may be the better procedure in case the centrifuge tube has too large a diameter at the tip. Next the sides of the tube are washed down with 2 c.c. of the saturated CaC_2O_4 solution in 0.5 per cent NH_4OH and the precipitate stirred up with a very fine glass rod. The rod is then rinsed off with 2 c.c. more of the 0.5 NH_4OH solution. The centrifuging is repeated in the same way, and so also the decanting. The washing and decanting is repeated twice more making a total of three washings. After the last decanting add 2 c.c. of the $N.H_2SO_4$, break up the precipitate with a fine glass rod, and then heat the tube for two minutes in a bath of boiling water preferably in a

*We have found, however, that N/100 $KMnO_4$ prepared from old stock N/10 $KMnO_4$ solution, and kept at ordinary room temperature, protected from light, dust and outside air changes value much more slowly than freshly prepared N/100 $KMnO_4$ solution.

white porcelain dish so as to give a good white background to help in detecting the end point. While keeping the mixture hot (not over 70° C.), titrate with the N/100 $KMnO_4$ solution by means of a microburette* graduated to $\frac{1}{100}$ c.c. and provided with a very fine point so as to deliver 100 drops or more per cubic centimeter. The first detectable pink color which remains for one minute after mixing is taken as the end point. Make a blank titration on 2 c.c. of $N.H_2SO_4$. Deduct this from your known titration, and calculate the milligrams of Ca per 100 c.c. of plasma. This is calculated by the formula:

$$\frac{(\text{Titration minus Blank}) \times \text{correction factor} \times 0.2 \times 100}{2}$$

or

(Titration minus Blank) × correction factor × 10 = mg. of Ca per 100 c.c. of plasma.

CONCLUSIONS

1. The most dependable results are obtained when the precipitated calcium oxalate is washed three times with a saturated solution of calcium oxalate in 0.5 per cent NH_4OH.

2. A microburette of the type mentioned is very convenient, and adds to the sensitiveness of the method.

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AN ACCURACY CHECK FOR METABOLISM APPARATUS

BY HORRY M. JONES, M.D., PH.D., CHICAGO, ILL.

WITH the wide increase, during the past ten years, in the use of the basal metabolic rate in medical practice, there has grown greater instead of less confusion regarding the accuracy of the various methods and devices now used for determining the basal metabolic rate.

A standard recognized procedure for testing the accuracy of a given method or device, directly in terms of known physical-chemical units and indirectly by comparing it with other devices whose accuracy has been similarly checked, could assist materially in clearing up this confusion and doubt regarding the accuracy of the various devices now available for making basal metabolic rate determinations.

The first very practical use for such a standard procedure would be, not so much for the purpose of checking the accuracy of the apparatus when received new from its manufacturer, but more especially for determining

*A microburette devised by one of us (F. C. Koch[4]) is a very convenient form of apparatus for this purpose. The reagent supply reservoir and connecting tube, painted black, insures a ready supply of reagent protected from light and dust. This burette is manufactured by Central Scientific Company, Chicago, Illinois.

whether or not the accuracy it possesses when new continues as time goes on. An apparatus may be accurate when first installed, but certain types of them rarely continue to remain so, for the reason that the causes of error are of such a nature that hardly a week goes by that one or the other of these sources of inaccuracy does not appear, their number and seriousness increasing as time goes on. In other words, it is not so important to test the original accuracy as it is to see if this accuracy continues.

A second most important use for such a standard procedure, if made simple enough for the average laboratory technician to understand and use, would be to serve as a stimulus to more painstaking technic in routine tests on the human subject. The too commonly careless or poorly trained technician needs a strict taskmaster. Any technician, whether well or poorly trained, knows that his metabolic rate determinations on the human patient must be accepted without question. There may be those who doubt the findings as stated in his report, but in the absence of other proof, too often his report, right or wrong, stands.

But imagine that same technician put to the task of proving an already proved fact of chemistry, namely, that a given quantity of alcohol requires its exact chemical equivalent of oxygen for its combustion. In other words, let him make a metabolism test on a device with a machine-made metabolic rate, a rate known to be within at least one-half of one per cent. The experiment challenges his best skill, and by compelling his strictest attention to details, develops in him, unawares, a practically flawless technic, itself a most essential requirement for accuracy in the ordinary routine of metabolic rate determination on the human subject as well.

A third very practical use for such a standard procedure is in teaching the theory and practice of the subject of metabolism to medical students. Practically every medical school in this country today provides somewhere in its curriculum opportunity for the student to become acquainted by personal experience with some device or other used for making the test.

The importance of attention to technical details, however, cannot be sufficiently impressed upon the student, for the reason that the human subject used for making the student demonstration test always has an unknown metabolic rate, varying from nothing to 100 per cent above the normal limits, depending upon his cooperation, emotional state and other factors introduced by the test subject. But the demonstration could be made with the accuracy of any other laboratory experiment, provided the student is required to make the test on a device of known metabolic rate, one not influenced by emotions, specific dynamic action of foods, lack of a sufficient period for rest and other known and unknown factors which, in the human test subject, are causes of enormous fluctuations in the metabolic rate above and below the normal.

The student, having performed his laboratory experiment, using the alcohol check apparatus for his test subject, will then be required to review the chemical and physical factors involved in the calculation of a metabolic rate, to calculate the caloric value of the liter of oxygen, to determine the respiratory quotient and its relation to the caloric value, and to compare this heat output with that of the average normal human subject.

This less complicated approach to the subject is made more readily understandable for the reason that in this experiment, only one fuel substance is involved, namely, alcohol, in the process of oxidation, instead of many substances in the oxidations going on in the human subject.

The principle of the check method here described, familiar to researchists in metabolism work, is the so-called "alcohol check."

According to the chemical equation representing the burning of ethyl alcohol,

$$C_2H_5OH + 3O_2 = 2CO_2 + 3H_2O,$$

one molecular weight of alcohol (46 gm.) requires for its combustion three molecular weights of oxygen, or 96 gm. (i.e., 67.2 liters) of oxygen calculated as dry gas at 0° C. and 760 mm. Hg. Therefore, one standard liter of oxygen burns up 46 ÷ 67.2, i.e., 0.684 gm. of absolute alcohol.

A measured volume of alcohol is burned in a closed chamber to which is connected the metabolism apparatus about to be tested. The gases of combustion as they form are "breathed" by a pump into the metabolism apparatus. The oxygen, supporting the combustion, being used to form CO_2 and H_2O, and these, in turn, being absorbed by the soda lime, is thus gradually removed from the metabolism apparatus, and it is the entire purpose of the test to show that the amount of oxygen so removed is the exact chemical equivalent of the alcohol burned, no more and no less.

The value of the alcoholic check lies almost wholly in finding out the many reasons why the apparatus does *not* "check." Gas leaks; soda lime exhausted, or perhaps water soaked; spirometer improperly counterbalanced, or perhaps dented on one side; guide lines on tracing sheets printed out of line; too little water in spirometer; rotted breathing valves, ineffectual or too resistant to the breathing; and many other unsuspected sources of inaccuracy are brought to light, to say nothing of the many sins of the operator himself.

The alcoholic check device consists essentially of a gas-tight glass chamber provided with means for intake of oxygen and outflow of CO_2; provided also with an alcohol burner so devised that it can be adjusted to different rates of alcohol flow. The quantity of heat generated by the flame, being approximately that given off by the average size adult, is dissipated by radiators, to eliminate errors in reading the oxygen volume, which would occur if the gases in different portions of the circuit vary in their temperatures at the conclusion of the test, an error which could not be eliminated by calculation, i.e., correcting for this temperature change by taking the temperature of any one part of the circuit of gases. The rise in temperature, within the alcohol check apparatus itself, at the conclusion of any one test over its temperature at the beginning of that test is thus reduced by these radiators to a point too insignificant to take into account.

The gases of combustion are alternately expired and inspired by means of a pump operated by an electric motor, the latter being adaptable, without change of any kind, to direct or to 25 to 60 cycle type of alternating current.

It is not necessary, or even advisable, to use absolute alcohol. From about 85 to 90 per cent alcohol tends to remain at a fairly constant concentration, with reasonable care in protecting it from contact with the air. The

percentage concentration of a given supply of alcohol may be determined by the specific gravity method, accurate to about one-tenth of 1 per cent.

Variations in temperature are the commonest causes of variations in the check determinations. For each 1° C. change in temperature, there will be a 0.1 per cent variation in the volume of alcohol, as measured by the ordinary pipette. Each 1° C. change in temperature will cause a 0.4 per cent variation in the volume of oxygen.

With these variations, due to temperature changes, taken into account, and with the rate of ventilation (respiration) set at a constant rate, other influences quickly become constants, after the first few seconds from the beginning of the experiment.

The tube feeding the alcohol flame is of such caliber that one may easily read the volume of alcohol accurate to within one-thousandth of a cubic centimeter.

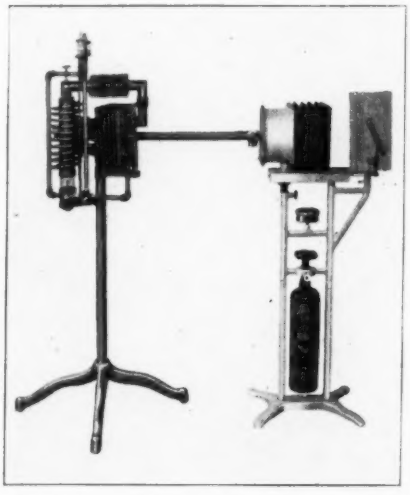

Fig. 1.—Alcohol check device connected to basal metabolism apparatus.

The basal metabolism apparatus attached to the alcohol check apparatus (see Fig. 1) is so calibrated that one may read the volume of oxygen accurate to within two one-thousandths of the standard liter of oxygen. The combined variations of both instruments will thus be not more than three parts out of one thousand, or three-tenths of 1 per cent.

It has been taught, and is commonly believed, that the alcohol check on a metabolism apparatus cannot be closer than about 3 per cent. This teaching is incorrect. It is possible to measure off a quantity of liquid (alcohol) to match up with its chemical equivalent of a gas (oxygen) with all the precision and exactness of any chemical experiment, and this should certainly be, and is, less than 3 per cent of the theoretical.

The results of check determinations made with this apparatus at the University of Illinois College of Medicine on various types of basal metabolism apparatus will be the subject of a future report.

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A STUDY OF THE PRESSOR METHOD FOR THE STANDARDIZATION OF PITUITARY EXTRACT*

By Edward E. Swanson, Indianapolis, Ind.

I. REVIEW OF LITERATURE

THE need of accurate methods of standardization of pituitary extract for its uterine activity (oxytocic) and blood pressure raising activity (pressor) demands more technical study of the methods; especially is it so since there has been considerable discussion and controversy as to whether the physiologic properties of the posterior portion of the pituitary gland are due to more than one principle. Dudley[1] found that continuous extraction of the purified pituitary solution with butyl alcohol at reduced pressure yields a residue that contains all the uterine principle and a part of the pressor principle. Later Dudley[2] gave evidence for the presence of at least three different physiologically active principles in the posterior gland. Führer[3] reported the isolation of four crystalline principles. Abel and Kubota,[4] Abel and Nagayvama[5] reported that the oxytocic-pressor actions of the posterior gland were due to one active specific substance or hormone. Abel and Rouiller[6] obtained results by a high purification of the infundibulin, which led them to believe that vasomotor, oxytocic, and renal activities are only the physiologic actions of one and the same hormone. Abel, Rouiller and Geiling[7] and Abel,[8] by still greater purification concluded that the oxytocic-pressor, diuretic-antidiuretic, and respiratory activities were properties of one and the same substance. Smith and McClosky[9, 10] found that the oxytocic, pressor and renal actions had the same diffusion rate through a series of collodion membranes of graded permeability, and quantitatively determined the ratio of renal activity of their standard powder as compared with the fresh gland extract to be the same as the oxytocic pressor ratio, that is to say, 1:7 weight for weight. These results led them to believe that the oxytocic-pressor-renal actions are the properties of one hormone. Hogben and Schlapp[11] found that the substance responsible for the consistent depressor action of the extract on the avine circulation is not the same principle which produces depression in the dog or cat, nor is it histamine. Hogben[12] later found that the substance present in the extract causing a powerful depressor action in the bird is a specific property of the fresh gland and appears to be associated like the pressor (mammalian) substance with the pars nervosa rather than the pars-intermedia. Hogben and deBeer[13] found that the extracts of the pars intermedia have a higher oxytocic value than pressor value, as compared with the extracts of the pars nervosa. Schlapp[14] verified Dudley's work of extraction with butyl alcohol, and found that the quantity of pressor and melanophore substance or substances, exceeded that of the oxytocic substance. He therefore concluded that the oxytocic response and pressor-

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Received for publication, June 28, 1928.

melanophore activities must be attributed to distinctly separate principles. However, there is no positive evidence that the pressor-melanophore actions are due to distinct substances.

The above controversy in regard to the theories of unitarian and multiple activities of the posterior gland has recently been reported by Kamm, Aldrich, Grote, Rowe and Bugbee[15] in favor of the multiple theory; these authors have separated chemically the oxytocic and pressor hormones, by purification and fractionation with solvents and precipitants and have quantitatively shown their separation by the oxytocic and pressor methods of assay.

The physiologic properties of the principles of the posterior body are easily destroyed or decomposed by strong acids, alkalies, trypsin, and heat. Abel and Kubota,[4] Abel and Nagayvama[5] heated extracts with HCl or boiled them in reflex condensors destroying completely the pressor principle but not all the oxytocic principle. Dudley and Dale and Schlapp later confirmed the same destructive action. Recently Stasiak[16] by boiling or fractional sterilization at various hydrogen-ion concentrations with HCl and acetic acid, found that the P_H controls the stability of the oxytocic property. This data would have been more conclusive if the pressor value had also been determined.

II. THE PARTIAL SEPARATION OF THE OXYTOCIC AND PRESSOR PRINCIPLES IN THE MANUFACTURE OF PITUITARY EXTRACT

In the manufacture of pituitary extract, which is the separation of the posterior portion from the anterior portion, the posterior portion is extracted with an acid water solution. This solution is purified by fractional precipitation, isoelectric precipitation, etc., until a pure extract is obtained. The assays of the different fractions or precipitates show by the oxytocic and pressor methods of standardization a distinct difference in the pressor and oxytocic action. The final solution of pituitary extract has a balanced oxytocic-pressor value by mixing the fractions or precipitates containing the various amounts of oxytocic and pressor activity as determined by the oxytocic and pressor methods of assay. During the assay of the different fractions a 1:2, 1:3 and even 1:5 pressor-oxytocic ratio and vice versa oxytocic-pressor ratio has been obtained in the regular purification process of manufacture of pituitary extract, thus verifying a part of the work of Kamm[15] and his associates.

III. THE TOLERANCE FACTOR

Hogben, Schlapp, and McDonald[17] in their excellent report on the pressor method state, "The results of our experiments have been to show that after a lapse of time, varying with the size of the dose given, full response can be recovered when complete immunity has been produced by repeated injections; and it became evident, at an early stage in the investigation, that by administering the extract only at the end of the period adequate for complete recovery, a constant response can be obtained over periods of many hours." This period of constant response they found to be one hour and the amount injected was approximately one-half of the dose that will give a maximum response. As Dale[18] suggested, the rate of excretion or destruction or establishment of immunity period, may depend upon the size of the dose given. If Hogben and

his coworkers had given injections at thirty minute intervals, the amount of pituitary extract injected would be less than is required for the hourly interval period. The increase in blood pressure or contractions would be less, thus at half hour intervals as well as at hourly intervals there would be a point of inflexion where the dp = dx is a maximum. To illustrate, in Chart 1, if a cat requires two hours to establish immunity, or a period of constant response, with an injection of 1 unit, one hour for a 0.5 unit injection, thus 0.25 unit injection at thirty minute intervals should establish a period of immunity with a sufficient margin of response to allow larger or smaller injections or units to be given to obtain a quantitative assay equal in accuracy to the one hour period. This plan has been used in the following study of the spinal and anesthetic cats in the standardization of pituitary extract by the pressor methods.

IV. THE INTERNATIONAL UNIT

The League of Nations (page 14 of a report by the Second International Conference on the Biologic Standardization of Remedies, Geneva, 1925) expressed the activity of a solution of pituitary extract in terms of international units each c.c. being equivalent to 10 international oxytocic units. The official solution of pituitary extract of the U.S.P. represents in each c.c. the equivalent of 5 mg. of a standard powder, which may be obtained from the U. S. Bureau of Chemistry. Each mg. of this standard powder contains 2 international oxytocic units. The U.S.P. solution of pituitary extract should contain 10 international oxytocic units, no requirement being made as to the pressor units. This standard powder was used in the study of the pressor method, 1 mg. being considered equal to 2 pressor units.

V. THE PRESSOR METHOD OF STANDARDIZATION

A. *Spinal Cat Method.*—Considering the above review and discussions of the literature, the definite proof of two principles in the posterior gland, the similarity in response to purification methods of these principles, and their possible injury by chemical reagents the need of accurate methods of standardization is indeed important. The oxytocic method has been extensively studied by Dale and Laidlow,[19] Dale and Burn,[20] Roth,[21] Pittenger,[22] Spaeth,[23] Eckler,[24] Nelson,[25] Hamilton and Rowe,[26] Hamilton,[27] Rowe,[28] and Swanson.[29] The pressor method has been reported by Hamilton and Rowe[26] and more recently by Hogben, Schlapp and McDonald.[17] These later workers describe a method on spinal cats, which is essentially that of the Dale and Laidlow[19] spinal cat method in studying the depressor and pressor properties of the pituitary gland. They have applied the spinal cat method as a quantitative method for determining the pressor value of pituitary extract. To briefly quote: "The cord is exposed under deep anesthesia by clipping off the neural arch of the axis vertebra, the aperture in which is plugged plasticine after transection of the cord and destruction of brain. No injection made until two or three hours after the operation. The animal should be kept warm by wrapping, a rectal temperature of 35° C. to 37° C. maintained. If properly inflated and special importance is to be attached to the degree of insufflation a cat prepared in this way will maintain a blood pressure of 80 mg. for twenty-four hours. Each in-

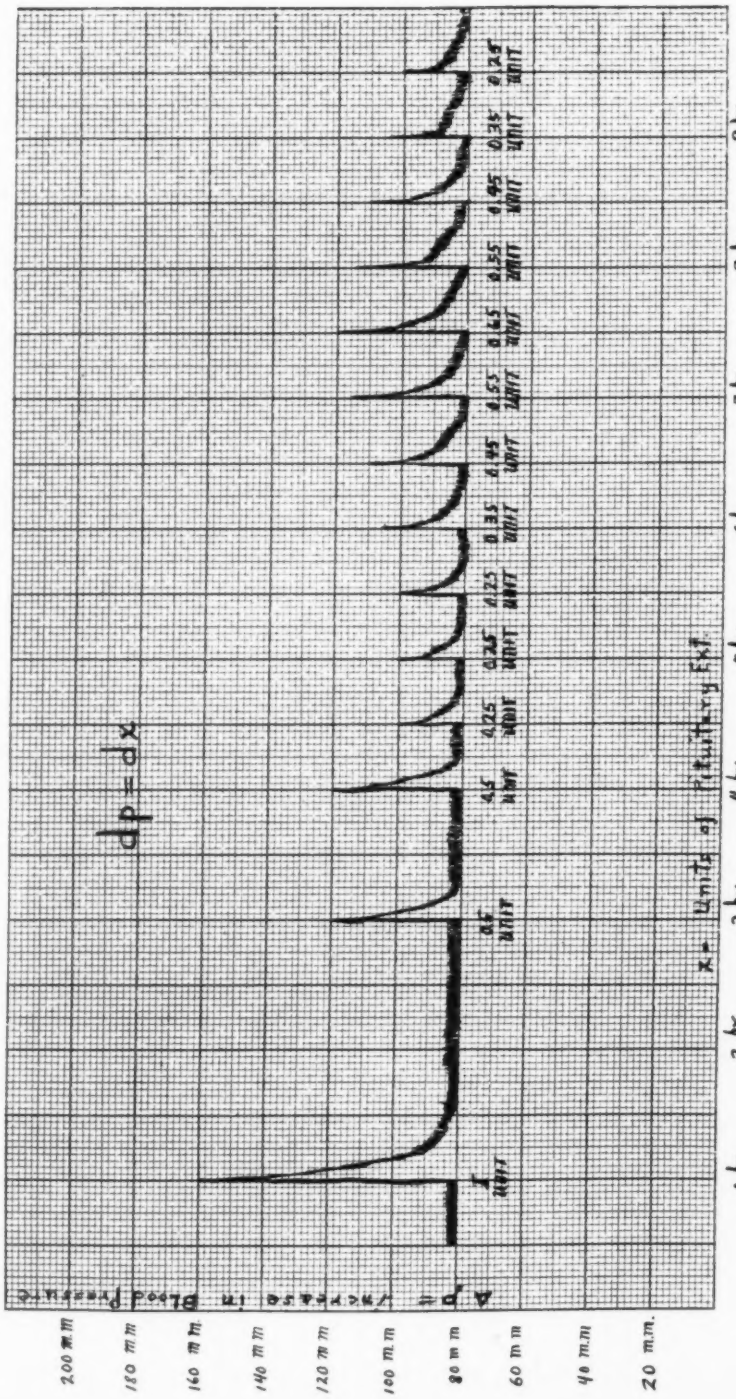
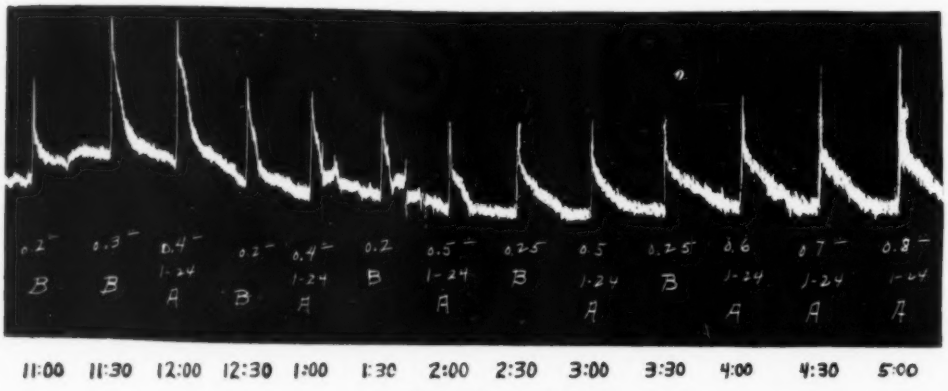

dp = dx
Blood Pressure in Millimeters of Mercury
200 m.m
180 mm
160 mm
140 mm
120 m m
100 m.m
80 m m
60 m m
40 m.m
20 m.m.
1 hr
2 hr
3 hr
4 hr
5 hr
6 hr
7 hr
8 hr
9 hr
x = Units of Pituitary Ext.
0.5 UNIT
0.25 UNIT
0.35 UNIT
0.45 UNIT
0.55 UNIT
0.65 UNIT
0.75 UNIT
0.85 UNIT
0.95 UNIT
0.35 UNIT
0.25 UNIT

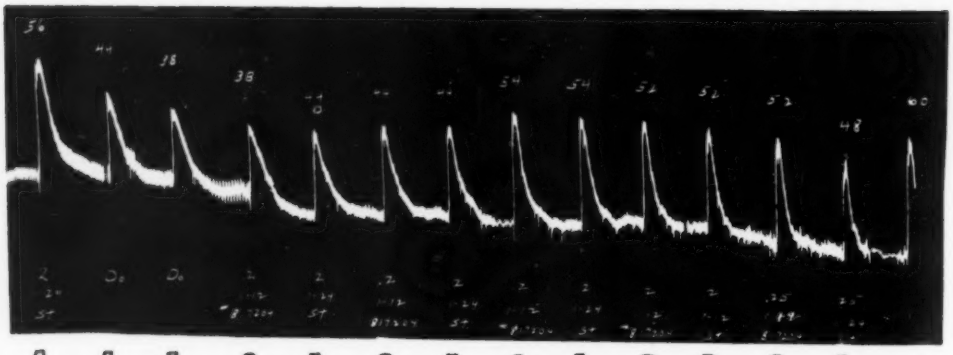
Chart 1.

jection of pituitary extract given at hourly intervals." This method, after one masters the technic of preparing a spinal cat, is indeed an excellent and accurate one. However for commercial laboratories where the period of work is only six to eight hours a day, considering that only one injection is given each hour, and two or three hours required before the spinal cat receives the first dose, only four or five injections can be given in a day, unless the operator



SPINAL CAT

FIGURE I



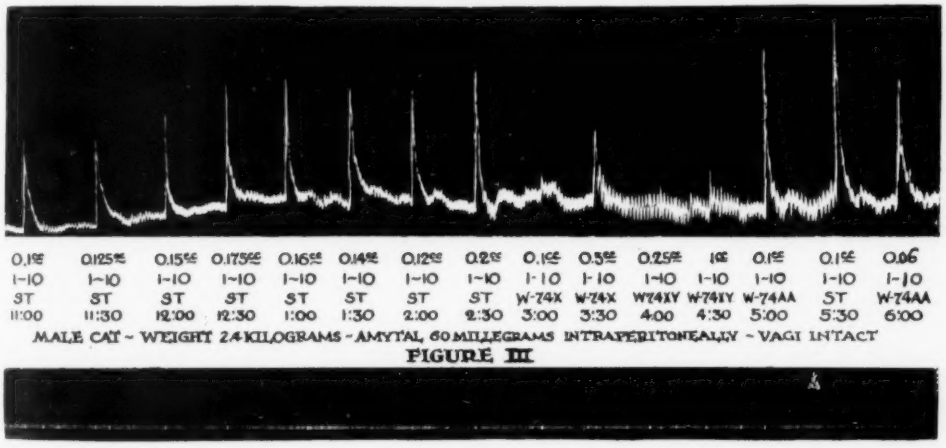
SPINAL CAT

FIGURE II

continues the injections into the hours of the remaining day and night. A successful spinal cat receives approximately only four or five injections in eight hours, which is not sufficient for a quantitative assay. In the study of this method the spinal cat maintains an excellent blood pressure level, and eliminates the anesthesia factor which is very discouraging at times. Furthermore, giving the injections at half hour intervals instead of at hourly intervals, providing the injections are not too large, it should make the spinal cat method more practical in the commercial testing of pituitary extract.

Fig. I represents one of many spinal cats with injections given every half hour. The first ten injections are alternating injections of Sample A (solution of pituitary extract containing 24 international oxytocic units and 24 pressor units); and Sample B (government standard powder No. 232 each mg. equivalent to 2 international oxytocic units and 2 pressor units and a prepared solution of this powder as directed by the U.S.P. so that each 1 c.c. contains 1 mg. of this powder). The remaining contractions are gradual 10 per cent increase in the injections of Sample A. This tracing shows an accuracy of 10 per cent variation in the amount of pituitary extract.

Fig. II represents a spinal cat with injections given every half hour. The injections are alternating doses of Sample A (24 oxytocic and pressor units) and Sample C (12 oxytocic and pressor units). The tracing shows that Sample C is one-half as active as Sample A and Sample A contains the equivalent of 12 mg. of Sample B (government powder) or 24 units (oxytocic and pressor).



MALE CAT - WEIGHT 2.4 KILOGRAMS - AMYTAL, 60 MILLIGRAMS INTRAPERITONEALLY - VAGI INTACT

FIGURE III

B. The Anesthetic Cat Method.—For commercial standardization the anesthetized dogs or cats are more practical, in that it is comparatively simple to produce or prepare an animal with anesthesia. There are many anesthetics that may be used, chloretone, paraldehyde, urethane, etc.; these substances produce a deep depressing anesthesia, with low blood pressure, which gives small contractions or increase in blood pressure by the repeated injections of pituitary extract. The injections of pituitary extract must be small, to give consistent quantitative increases or contractions in the blood pressure. Too large doses of extract will develop tolerance with a gradual decrease in response or contractions and later a depressor action. More recently the barbital series as the sodium salt have been used as an anesthetic in pharmacologic study. These compounds do not give such a depression on the heart and blood pressure as compared to chloretone, etc.

The following technic has been used with comparatively good results in the standardization of pituitary extract by the pressor method. The cat is weighed, injected intraperitoneally with 60 mg. of amytal (isoamylethyl barbituric acid) prepared in solution as the sodium salt, as follows: 1 gm. of

amytal is added to 8.85 c.c. $\frac{n}{2}$ NaOH, heated to 80° C. for fifteen minutes, diluted to 10 c.c. with distilled water, and finally filtered. *This solution should be made fresh whenever needed.* The injection of 60 mg. of amytal as the sodium salt per kg. will produce anesthesia in thirty minutes. Some cats require more than 60 mg., but the average dose is approximately 60 mg. per kg. Following the exposure of the carotid artery, femoral vein, and the adjustment of cannula and manometer, the reflexes should be observed. Page and Coryllos,[30] Swanson and Page,[31] and Edwards and Page[32] found that certain reflexes will indicate the degree of anesthesia. The sensory reflexes should be absent and only a slight corneal or conjunctival and knee jerk reflexes may be present. The blood pressure will maintain an approximate height of 100 mm. to 120 mm. The injections of pituitary extract are given at half hour intervals,

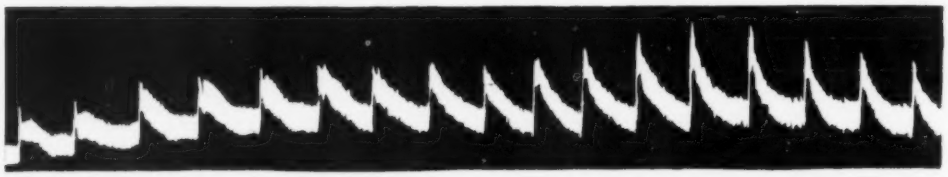


FIGURE IV

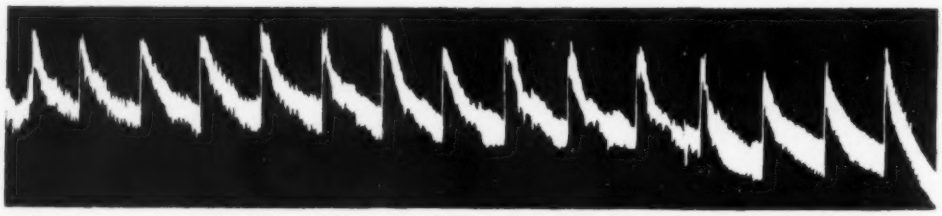


FIGURE V

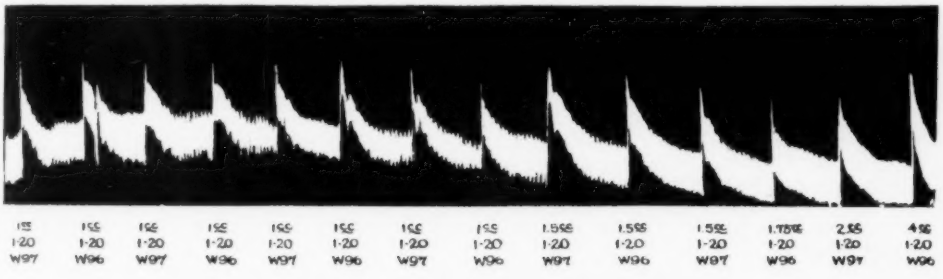
dilutions of 1 to 10 or 1 to 20 of a 10 international oxytocic unit solution preferred, the average dose being approximately 0.1 unit to 0.5 units depending upon the sensitiveness of the cat.

The following tracings represent a comparative assay of samples of pituitary extract prepared in our laboratories. Some of the samples represent a lot of pituitary extract in the process of manufacture, other samples represent dilutions of a known standardized solution, their dilutions or strength being unknown to myself.

Fig. III is a very sensitive cat under anesthesia. The first eight injections are the standard solution (10 international oxytocic units per 1 c.c.) of 0.10, 0.125, 0.15, 0.175, 0.16, 0.14, 0.12, and 0.2 units respectively. These various amounts of units show a quantitative response in the increase of the blood pressure. W-74X, W-74XY, W-74AA are samples of certain fractions in the

process of a manufactured lot of pituitary extract. Fractions W-74X and W-74XY show far less response to increases of the blood pressure than W-74AA, W-74AA being approximately equal to the standard.

Fig. IV represents the tracing of a cat (amytal anesthesia). This cat is not so sensitive as the cat in Fig. III requiring 0.5 units of the standard to produce consistent contractions. Sample A_1 is a solution of pituitary extract that is stronger than the standard. The first eight injections are alternating injections of the standard and Sample A. The amount of Sample A_1 increased until 0.2, (1 to 10) equals 0.5 pressor units of the standard. Therefore, Sample A_1 contains about 25 pressor units. The remaining nine injections are increasing



MALE CAT WEIGHT 2.3 KILOGRAMS, AMYTAL, INTRAPERITONEALLY, VAGI CUT

FIGURE VI

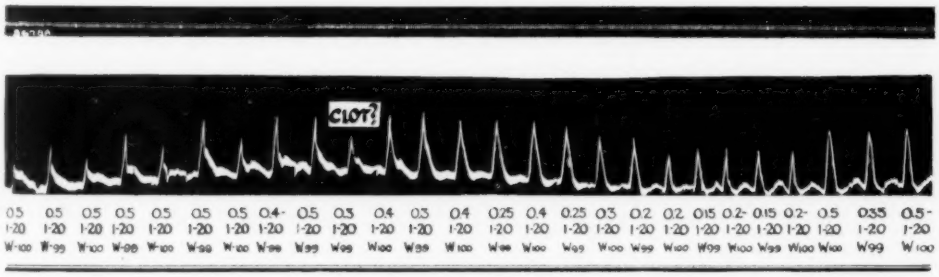


FIGURE VII
FEMALE CAT - WEIGHT 3 KILOGRAMS - VAGI CUT
AMYTAL INTRAPERITONEALLY

and decreasing amounts of the standard 0.6, 0.7, 0.8, 0.9, 1, 0.9, 0.8, 0.7, and 0.6 units respectively. These injections show a 10 per cent variation in response of contraction, and that it is possible to assay a pituitary extract solution by the pressor method within an accuracy of 10 per cent.

Fig. V represents the tracing of a cat (amytal anesthesia) that is very sensitive. Samples A_2 and A_3 are standard powders prepared by myself according to the method of the U.S.P. and the U. S. Bureau of Chemistry. These samples were compared with a Government standard powder No. 232 obtained from Dr. Munch of the Bureau of Chemistry. The tracing shows that Samples A_2 and A_3 are equal to the Government Standard Powder or each mg. of powder is equivalent to 2 pressor units.

Fig. VI represents the tracing of a cat (amytal anesthesia) vagi cut. W-96 and W-97 are samples of pituitary extract, their strength being unknown to

me. Alternating injections of 1 c.c. (1 to 20) of W-96 and W-97 gave equal responses in the rise of blood pressure, therefore W-96 being equal to W-97. It was learned later that each sample was taken from the same lot of pituitary extract solution containing 10 international oxytocic units.

Fig. VII represents the tracing of a cat (amytal anesthesia) vagi cut. W-99 and W-100 are samples of pituitary extract, their strength being unknown to me. Twenty-six alternating injections were given to this cat. Sample W-100 assayed 60 per cent to 70 per cent of W-99. The dilutions were made so that W-100 would be 66⅔ per cent as active as W-99 which was a regular manufactured lot of pituitary extract containing 10 international oxytocic units.

Table I represents the results of the assayed samples, their strength in units both pressor and oxytocic values as determined by the pressor and oxytocic methods.

TABLE I

PREPARATION NO.	MADE BY	PRESSOR UNITS	INTERNATIONAL OXYTOCIC UNITS	KNOWN PRESSOR-OXYTOCIC UNITS
A Mfg. lot	Walden	24	24	24
A_1 Mfg. lot	Walden	25	25	--
A_2 St. Powder	Swanson	1 mg. = 2 units	1 mg. = 2 units	--
A_3 St. Powder	Swanson	1 mg. = 2 units	1 mg. = 2 units	--
B 232 Powder	Govt.	1 mg. = 2 units	1 mg. = 2 units	1 mg. = 2 units
C Mfg. lot	Walden	12	12	--
W-97 Mfg. lot	Walden	12	12	12
W-96 Mfg. lot	Walden	12	12	12
W-100 Mfg. lot	Walden	7.8	7.8	8
W-99 Mfg. lot	Walden	12	12	12

DISCUSSION

A discussion and study of the spinal and anesthetic methods of the standardization of the pressor principle of pituitary extract leads one to believe that either method is practical provided that injections are given at one-half hour intervals. The spinal cat is an excellent method, which eliminates the anesthetic factor, maintains a constant blood pressure level, and is very sensitive to injections of pituitary extract with distinct measurable contractions. The anesthetic cat is more practical in that the technic is more simple, and the anesthetic factor can be so regulated as to maintain an experiment that is as accurate as the spinal cat and will give an accuracy of 10 per cent variation.

Two samples of standard desiccated powder A_2 and A_3 prepared according to the U.S.P. assayed equal to a standard powder No. 232 received from the Bureau of Chemistry, each mg. containing 2 pressor units by the pressor method of assay.

A few samples of pituitary extract, their strength being unknown to the operator, were tested by the pressor method, and an approximately accurate assay was obtained that agreed with the dilutions and strengths known to the individual who made the preparations.

The technic of producing a spinal or anesthetic cat requires care in preventing clots in the cannula which should be thoroughly cleaned, boiled in acid or cleaning solution inserted into the artery in such a way as to prevent any dirt or hairs coming in contact with the inner surface of the cannula.

CONCLUSIONS

1. The partial separation of the oxytocic and pressor principles has been verified in the regular manufacture of pituitary extract.

2. Spinal and anesthetic cats are both accurate methods of determining the pressor principle in units, the anesthetic method being more practical in that the technic is simple.

3. Several samples of pituitary extract were assayed by the spinal and anesthetic methods. The tests show considerable accuracy by these methods.

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A SIMPLE PRECIPITATION TEST FOR SYPHILIS WITH SMALL QUANTITIES OF DEFIBRINATED FINGER BLOOD*

Preliminary Report

By B. S. Kline, M.D., Cleveland, Ohio

THE simple precipitation test for syphilis with a few drops of finger blood, described in this article, is as specific and as sensitive as the microscopic slide precipitation tests and the Wassermann test with the same antigen. Because it requires but a small quantity of comparatively easily obtainable blood, the finger blood precipitation test is particularly useful in the diagnosis of syphilis in infants and children. After its reliability is established by a sufficient number of comparative tests, finger blood may be used routinely in the diagnosis of syphilis.

Finger blood in small quantities, as is well known, is generally employed in hemoglobin determinations, red blood cell and white blood cell counts, and in certain agglutination tests.

A rapid and simple hemagglutination test with small quantities of whole blood obtained from the finger, for use in testing donors for transfusion, was reported by Rous and Turner[1] in 1915. For the test, a small quantity of blood is drawn from the finger of the patient into a Wright pipette containing 10 per cent sodium citrate. Blood is similarly obtained from the prospective donor. Two mixtures of these bloods are made: (a) 9 parts citrated blood of the patient to one part of citrated blood of the donor; (b) one part citrated blood of the patient to one part of citrated blood of the donor. The mixtures are made in Wright pipettes (from pipette to slide or Widal plate and back a number of times), and read two to fifteen minutes after preparation.

Whole blood obtained from the finger has been extensively employed in the Widal reaction since 1896 when Wyatt Johnson[2] reported its reliability for the detection of typhoid agglutinins. In this method, single drops of blood are collected on glass, cardboard, or paper and subsequently (as late as forty-four days) diluted with sterile water. One drop of the whole blood solution and one drop of a suspension of typhoid bacilli are mixed on a cover slip and this is inverted and sealed over a chamber. Readings are made fifteen minutes to one hour after preparation.

An ideally simple yet thoroughly reliable finger blood test for agglutinins was introduced by Bass and Watkins[3] in 1910. In this test approximately one-fourth of a drop of blood is spread on a microscopic slide (with the end of another slide). Immediately after making the spread, or sometime after the blood has dried, one drop of water is allowed to fall on the slide and is spread over the surface by a toothpick. As soon as the blood has dissolved (less than one minute), one drop of a suspension of typhoid bacilli is added, the mixture is agitated by tilting the slide from side to side for two minutes, and the reac-

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tion then noted. The use of an open microscopic slide and the employment of agitation to accelerate the reaction are advantages of simplicity and rapidity not possessed by the other Widal tests.

No reference to a test for syphilis with small quantities of defibrinated blood has been found in the literature.

MATERIALS FOR THE FINGER BLOOD PRECIPITATION TEST FOR SYPHILIS (SEE FIGS. 1 AND 2)

1. Micro Culture Slides (Will Corporation No. 11802), 3 × 1 inch with wells 16 mm. × 3 mm. for collecting the finger blood.

2. Toothpicks with flat ends for defibrinating the blood in the chambers.

3. Humidor (box with tightly fitting lid containing wet blotter) to keep the defibrinated blood until tested.

4. Microscopic slides, 3 × 2 inches, each with two double rings made as follows: A 3 × 2 inch microscopic slide is covered on both sides with Bon Ami paste. After the paste has dried (in a few minutes), it is rubbed off with a clean soft muslin cloth leaving a clean smooth surface. Upon the clean slide, toward one end, an iron harness ring 1¼ inches inside diameter 3/16 of an inch thick (obtainable at hardware stores) is placed. In its center a No. 13 cork or a No. 6 rubber stopper is placed. The well between them is filled with hot wax (two parts of ordinary vaseline and one part of parowax) from a 10 c.c. glass syringe. After the mixture cools a few minutes, the cork and ring are removed and the process repeated at the other end of the slide. Rings made with the mixture given above adhere well to the slide, whereas rings of parowax alone occasionally separate from the slide at low room temperatures and allow the fluid to escape.

A better method of making the outer rings is as follows: Upon the clean slide a steel mold 3 × 2 × ⅛ inches with two central wells 3.4 cm. in diameter is placed. A metal disc 1⅛ inches in diameter and 3/16 of an inch thick is then placed in the center of each well. The space between them is filled with hot wax from a 10 c.c. glass syringe. After the mixture cools a few minutes, the mold is removed by inserting a thin blade between it and the slide. After the outer rings are made (3.4 cm. outside diameter and 2.9 cm. inside diameter), the inner rings (15 mm. inside diameter) are made with hot parowax by means of a thread wound wire loop, 16 mm. inside diameter, devised by Green.[4]

5. Slide holders (for three 3 × 2 inch slides). The slide holder is a wooden lid of a slide box (3½ × 6¾ inches) containing an easily fitting thin wooden shelf having a small handle at each end made with a wire paper clamp (about one-third of the clamp is fastened under the shelf with adhesive tape and the remainder after being forced through the shelf is bent to serve as a handle).

6. Holder for two slide holders consists of a wooden lid (13⅝ × 3⅝ inches inside measurements, 14½ × 4 inches outside measurements) making it possible to use six slides at one time.

7. Ordinary 1 c.c. pipettes (graduated in hundredths) or 0.02 c.c. pipettes (graduated in thousandths) for pipetting the defibrinated blood.

8. Wright pipettes for delivering drops 0.015 to 0.016 c.c. of 7 per cent sodium chloride solution, and others to deliver drops 0.017 to 0.018 c.c. of

antigen emulsion. These pipettes may be made from ordinary glass tubing (10 mm. outside diameter) or from portions of 10 c.c. pipettes by drawing out one end into a fine tube of the proper diameter. A rubber bulb is used to control the pipetting. If a calibrated pipette is used, the quantity of the drop can be determined readily. If ordinary tubing is used, the following method of determining the proper sized drop is employed: 0.5 c.c. of 7 per cent sodium chloride solution or 0.5 c.c. of antigen emulsion is pipetted into a small container. The fluid is quickly drawn up into the pipette to be tested. The pipette is held vertically and slight pressure is exerted on the bulb to force drops out slowly. A satisfactory pipette for the antigen emulsions will deliver 27 to 29 drops per 0.5 cc., and a satisfactory pipette for the 7 per cent sodium chloride solution will deliver 31 to 33 drops per 0.5 c.c.

9. Antigen emulsions. The antigen employed is one described previously.[5] Briefly the details of its preparation are as follows:

Two hundred grams of dried heart powder (Difco) is placed in a two liter Erlenmeyer flask. One liter of absolute ethyl alcohol (99 plus per cent) is added.

After the flask is stoppered with a cork covered with tin foil, it is shaken vigorously by hand for from twenty to thirty minutes. (This short extraction removes almost all of the desired antigenic substance in the powder.) A shaking machine may be employed for the extraction. After a few hours in the machine practically all the antigenic substance is extracted.

The extract is filtered into a liter cylinder through good grade filter paper of medium texture (Schleicher and Schull No. 597, 38 cm.).

During filtration, the mixture is stirred with a wooden tongue depressor and toward the end, pressed with a cork until the powder is quite dry.

The extract (about 775 c.c.) is placed in the refrigerator at 8° to 10° C. for from fifteen to forty-eight hours.

During this time, a fairly heavy white precipitate settles out. This is filtered off and the filtrate, in a large evaporating dish, is concentrated on a water-bath or by an air heater (bathroom heater) at 45° to 50° C. determined by a thermometer bulb within the extract. As the alcoholic extract evaporates down, there is an irregular festoon at the periphery until the desirable concentration (about $\frac{1}{20}$ of the original volume) is reached. At this time the festoon disappears and the margin of the concentrated extract is sharp. The extract is now ready to be poured into acetone.

The extract is poured into 500 c.c. of acetone c. p. at 50° C. in a large evaporating dish. This is then placed in an air incubator at 37° C. until the temperature of the fluid reaches this level. After this it is allowed to stand at room temperature for several minutes when the acetone is decanted leaving a soft yellow brown wax-like substance adherent to the dish. The lipid is then worked together and placed in a glass stoppered bottle with 80 c.c. of absolute ethyl alcohol (99 plus per cent) at about 50° C.

After a few minutes' shaking, the bottle is placed in an oven (at 50° to 56° C.) for thirty minutes to dissolve as much of the lipid as possible.

On removing the bottle from the oven it is shaken for a few minutes and then placed in the refrigerator at 8° to 10° C. for forty-five minutes.

The solution is then filtered and the filtrate is evaporated down at 45° to 50° C. (water-bath or air heater) resulting in a soft brown wax-like substance (antigen lipid). The antigen lipid is weighed and to each gram, in a glass-stoppered bottle, 10 c.c. of absolute ethyl alcohol (99 plus per cent) is added. After the bottle is shaken for a few minutes it is placed at 50° to 56° C. for thirty minutes, and then shaken a few minutes. (The lipid although obtained from a clear alcoholic solution is again incompletely soluble in alcohol.)

The slightly turbid solution is placed at 8° to 10° C. for an hour, and then filtered. The resultant clear filtrate is the antigen and contains about 8.75 per cent of the alcohol-treated acetone-insoluble lipid. (This lipid, if repeatedly treated with alcohol or precipitated with acetone, is still incompletely soluble in alcohol.)

The antigen is kept at room temperature.

With the antigen described above, emulsions may be prepared having the following advantages over Kahn antigen dilutions:

Antigen Emulsions	Kahn Antigen Dilutions
(a) Relatively stable, satisfactory for at least three days.	(a) Unstable, satisfactory for an hour or an hour and a half at most, usually less.
(b) Satisfactory at low room temperatures as well as at ordinary ones.	(b) Majority unsatisfactory at low room temperatures.
(c) The very sensitive emulsion is more sensitive than the Kahn antigen dilution and no less specific.	
(d) The emulsions are more uniform in quality and quantity than the Kahn antigen dilutions.	

The formula for the finger blood precipitation test antigen emulsion is as follows:

0.85 c.c. Distilled Water.
0.5 c.c. 1 per cent Cholesterin (C. P. Pfanstiehl) solution in absolute (99 plus per cent) ethyl alcohol.
0.1 c.c. Antigen.
2.95 c.c. 0.85 per cent sodium chloride (Merck c. p. or reagent) solution.

The emulsion is prepared in the following manner:

Into a one-ounce bottle 0.85 c.c. distilled water is pipetted. The bottle is held at an angle and the 0.5 c.c. of 1 per cent cholesterin solution is allowed to run along the side of the bottle.

The bottle is gently rotated from the neck for from fifteen to twenty seconds.

The bottle is held at an angle again and 0.1 c.c. of antigen is pipetted against the side from a 0.2 c.c. pipette (graduated in thousandths).

The bottle is promptly stoppered with a cork and shaken fairly vigorously (the fluid thrown from bottom to cork and back) for one minute.

Lastly, the 2.95 c.c. of 0.85 per cent sodium chloride solution is allowed to run in quite rapidly, the bottle is stoppered again and shaken as previously for one minute.

The very sensitive antigen emulsion for the finger blood precipitation test consists of the emulsion just described, treated in the following manner: About 1 c.c. of sensitive antigen emulsion is placed in a rack in a large pan or water-bath with the water at least two inches above the level of the emulsion. The water is maintained at 50° C. After twenty minutes at this temperature,

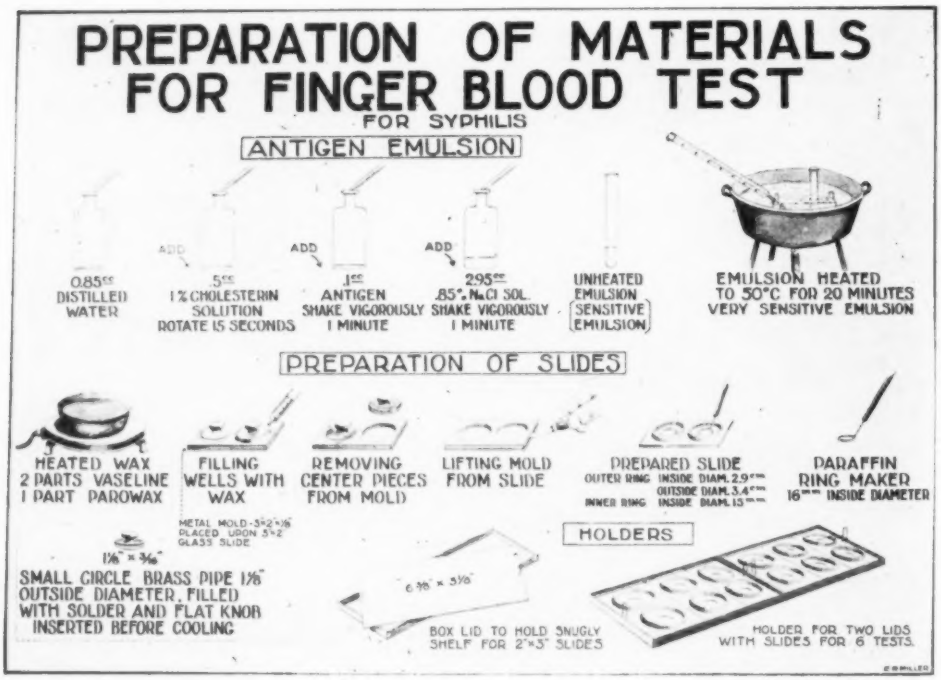
PREPARATION OF MATERIALS
FOR FINGER BLOOD TEST
FOR SYPHILIS
ANTIGEN EMULSION
ADD
ADD
ADD
0.85cc DISTILLED WATER
5cc 1% CHOLESTERIN SOLUTION ROTATE 15 SECONDS
1cc ANTIGEN SHAKE VIGOROUSLY 1 MINUTE
2.95cc .85% NaCl SOL. SHAKE VIGOROUSLY 1 MINUTE
UNHEATED EMULSION SENSITIVE EMULSION
EMULSION HEATED TO 50°C FOR 20 MINUTES VERY SENSITIVE EMULSION
PREPARATION OF SLIDES
HEATED WAX 2 PARTS VASELINE 1 PART PAROWAX
FILLING WELLS WITH WAX
METAL MOLD-3"x2"x1/8" PLACED UPON 3"x2" GLASS SLIDE
REMOVING CENTER PIECES FROM MOLD
LIFTING MOLD FROM SLIDE
PREPARED SLIDE
OUTER RING INSIDE DIAM. 2.9cm
OUTSIDE DIAM. 3.4cm
INNER RING INSIDE DIAM. 1.5cm
PARAFFIN RING MAKER
16mm INSIDE DIAMETER
1/8" x 3/16"
SMALL CIRCLE BRASS PIPE 1/8" OUTSIDE DIAMETER, FILLED WITH SOLDER AND FLAT KNOB INSERTED BEFORE COOLING
HOLDERS
6 3/16" x 5 1/16"
BOX LID TO HOLD SNUGLY SHELF FOR 2"x3" SLIDES
HOLDER FOR TWO LIDS WITH SLIDES FOR 6 TESTS.

Fig. 1.

the emulsion is removed and kept at room temperature until used (one-half hour to two hours after the original preparation of the emulsion).

The 1 per cent cholesterin solution for the antigen emulsion is prepared as follows:

To 1 gram of cholesterin (e. p. Pfanstiehl) 100 c.c. of absolute ethyl alcohol (99 plus per cent) is added and the bottle placed in an incubator at 50° C. to 56° C. The bottle is shaken at fifteen-minute intervals to promote solution which is complete in about forty-five minutes. This stock solution is kept in the incubator at 37° C. and is perfectly satisfactory for the preparation of the emulsion for as long as two months.

10. Defibrinated finger blood. The patient's finger tip is cleaned with alcohol and one or two puncture wounds made with a sharp needle. With the patient's hand in a dependent position, the bleeding finger is squeezed with

both hands and the blood allowed to fall into the chamber of a micro culture slide resting upon a chair or other object. The blood in the chamber is then stirred with the flat end of a toothpick until defibrinated (several minutes). The slide is next labelled with a china marking pencil, placed in the humidor and kept (in the ice chamber at about 10° C. preferably) until the blood is to be tested (from a few minutes to twenty-four hours after collection).

FINGER BLOOD PRECIPITATION TEST FOR SYPHILIS

(SEE FIG. 2)

1. Pipette 0.04 c.c. of defibrinated finger blood obtained as described above into each inner ring of the 3 × 2 inch slide.

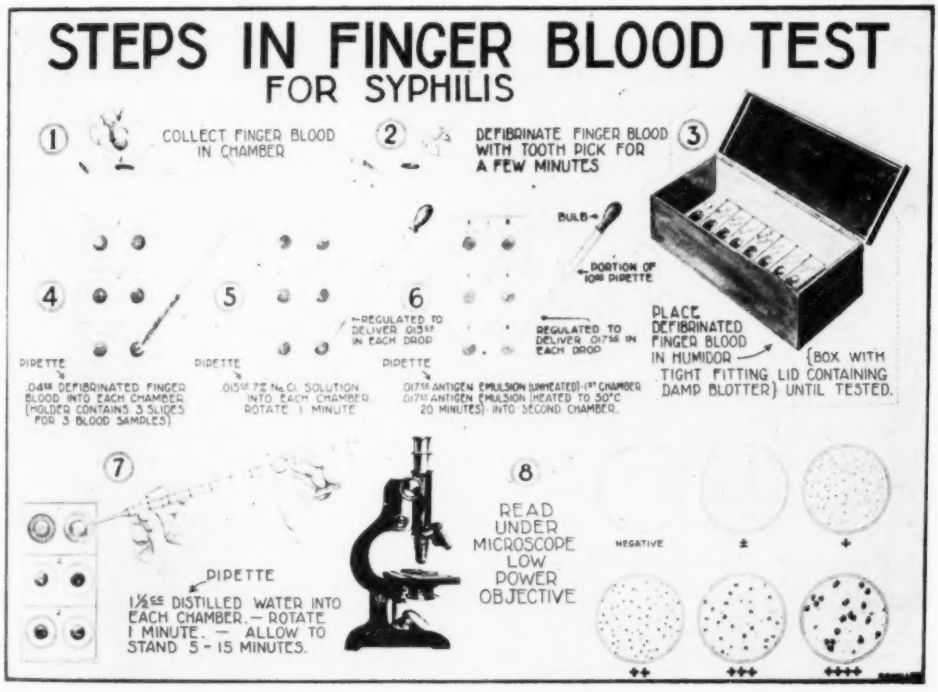
STEPS IN FINGER BLOOD TEST
FOR SYPHILIS
1
COLLECT FINGER BLOOD IN CHAMBER
2
DEFIBRINATE FINGER BLOOD WITH TOOTH PICK FOR A FEW MINUTES
3
BULB
PORTION OF 10m PIPETTE
PLACE DEFIBRINATED FINGER BLOOD IN HUMIDOR
(BOX WITH TIGHT FITTING LID CONTAINING DAMP BLOTTER) UNTIL TESTED.
4
5
6
REGULATED TO DELIVER .015cc IN EACH DROP
REGULATED TO DELIVER .017cc IN EACH DROP
PIPETTE
.04cc DEFIBRINATED FINGER BLOOD INTO EACH CHAMBER (HOLDER CONTAINS 3 SLIDES FOR 3 BLOOD SAMPLES)
PIPETTE
.015cc 7% Na Cl SOLUTION INTO EACH CHAMBER ROTATE 1 MINUTE
PIPETTE
.017cc ANTIGEN EMULSION (UNHEATED) 1st CHAMBER
.017cc ANTIGEN EMULSION (HEATED TO 50°C 20 MINUTES) INTO SECOND CHAMBER.
7
PIPETTE
1½cc DISTILLED WATER INTO EACH CHAMBER. — ROTATE 1 MINUTE. — ALLOW TO STAND 5 - 15 MINUTES.
8
READ UNDER MICROSCOPE LOW POWER OBJECTIVE
NEGATIVE
±
+
++
+++
++++

Fig. 2.

2. Allow one drop (0.015 to 0.016 c.c.) of 7 per cent sodium chloride solution to fall (from a pipette described above) into each inner ring containing blood, and rotate the slide quite vigorously for one minute.

3. Allow one drop (0.017 to 0.018 c.c.) of sensitive antigen emulsion to fall (from a pipette described above) into the first blood salt mixture. In the same manner, allow a similar quantity of very sensitive antigen emulsion to fall into the second blood salt mixture. Rotate the slide with moderate vigor for four minutes. (With proper sized rings and proper rotation, spilling from the inner chamber with consequent spoiling of the test will not occur.)

4. Allow 1.5 c.c. of distilled water to fall (from a 15 c.c. pipette) into each mixture (the mixtures spill over to the outer rings) and rotate the slide gently

for one minute to completely lake the blood. Allow the slide to stand from five to fifteen minutes (ten minutes preferably) before reading.

5. Readings are made through the microscope at a magnification of about one hundred times (low power objective 16 mm., eyepiece 12 X or 12½ X. A 10 X eyepiece is not as satisfactory.) The light should be somewhat brighter than for reading the microscopic slide precipitation test with serum. The contents of the chambers are examined in different portions and at different levels, and read negative if clumps are absent and ± to + + + + depending upon the size of the clumps and upon the degree of the clumping (see Fig. 2). Leucocytes and any débris which may be present can be readily differentiated from the compact refractile yellow brown clumps of a positive reaction. If large clumps are preesnt they may all be at the bottom of the fluid. Ordinarily, however, the clumps are quite evenly distributed in all levels. The negative tests contain granules but no clumps. To differentiate these granules from the small clumps of a ± test, various portions are carefully examined at different levels by turning the large micrometer screw of the microscope very slowly.

Six blood specimens may be tested with the sensitive emulsion and with the very sensitive emulsion at one time by using a holder (see Fig. 1) containing two lids each of which carries three slides on a shelf.

At the time blood was obtained from a finger for the test just described, a larger quantity was drawn from an arm vein to furnish serum for the microscopic slide precipitation tests and for the Wassermann test. Fifty and four-tenths per cent of the finger bloods examined were obtained from luetic patients under treatment. Since the majority of these bloods gave ± to + + + + results, an excellent opportunity was afforded for a comparison of the sensitivity of the different tests. No false positive results occurred in any of the tests. The comparative results of the four tests with the same antigen are given in Tables I and II.

TABLE I

COMPARISON OF SENSITIVE FINGER BLOOD PRECIPITATION TEST FOR SYPHILIS WITH SENSITIVE SERUM TESTS

	FINGER BLOOD TEST AND SLIDE PRECIPITATION TEST (HEATED SERUM)[6]		FINGER BLOOD TEST AND SLIDE PRECIPITATION TEST (RAW SERUM)*		FINGER BLOOD TEST AND WASSERMANN TEST (CLEVELAND METHOD) (HEATED SERUM)	
	TESTS	PER CENT	TESTS	PER CENT	TESTS	PER CENT
Agreement	319	82.64	127	88.81	215	76.24
Relative agreement	64	16.58	16	11.19	47	16.67
Agreement or relative agreement	383	99.22	143	100.00	262	92.91
Disagreement	3	0.78	0	0	17	6.03
Anticomplementary					3	1.06
Total	386		143		282	

17 Disagreements with Wassermann Test: 12 False negative Wassermann Tests; 5 False negative Finger Blood Precipitation Tests.

3 Disagreements with Slide Precipitation Test (Heated Serum): 3 False negative Finger Blood Precipitation Tests.

Evaluation according to the method of Kahn:
Positive Reaction + + +, + + + and + +.
Doubtful Reaction + and ±.
Agreement, positive, negative or doubtful by both methods.
Relative agreement, positive or negative by one method and doubtful by the other.
Disagreement positive by one method and negative by the other, and vice versa.

*Slide precipitation test with raw serum to be described.

Tables I and II show that the finger blood precipitation test for syphilis is as specific and as sensitive as the microscopic slide precipitation tests and the Wassermann test of serum obtained by venipuncture.

TABLE II

COMPARISON OF VERY SENSITIVE FINGER BLOOD PRECIPITATION TEST FOR SYPHILIS WITH VERY SENSITIVE SERUM TESTS

	FINGER BLOOD TEST AND SLIDE PRECIPITATION TEST (HEATED SERUM)		FINGER BLOOD TEST AND SLIDE PRECIPITATION TEST (RAW SERUM)		FINGER BLOOD TEST AND WASSERMANN TEST (CLEVELAND METHOD) (HEATED SERUM)	
	TESTS	PER CENT	TESTS	PER CENT	TESTS	PER CENT
Agreement	92	83.64	66	83.54	77	76.24
Relative agreement	18	16.36	13	16.46	17	16.83
Agreement or relative agreement	110	100.00	79	100.00	94	93.07
Disagreement	0	0	0	0	6	5.94
Anticomplementary					1	0.99
Total	110		79		101	

6 Disagreements with Wassermann Test: 4 False negative Finger Blood Precipitation Tests, 2 False negative Wassermann Tests.

DISCUSSION OF THE FINGER BLOOD PRECIPITATION TEST FOR SYPHILIS

The principles underlying the Finger Blood Precipitation Test for syphilis are in part similar to those described by Kahn[7] for precipitation tests on heated serum. The following additional principles underlie the test with unheated blood: (1) Determination of the optimal quantity of electrolytes to secure satisfactory sensitivity. (2) Use of heat to increase the sensitivity of the antigen emulsion.

TABLE III

INFLUENCE OF ELECTROLYTES ON THE PRECIPITATION REACTION

DEFIBRINATED BLOOD NO.	NO SODIUM CHLORIDE SOLUTION ADDED	ADDED TO EACH BLOOD, BEFORE ANTIGEN EMULSION, ABOUT 0.013 TO 0.015 C.C. OF SODIUM CHLORIDE SOLUTION:										
		2%	3%	4%	5%	7%	8%	10%	12%	15%	16%	25%
1	−					++						
2	−					+++						
3						++++						
4							++++	++++		++++		+++, ++++
5							++	++		+, ++		+
6				±, +			+, ++					
7			±	±	++		++			+, ++		±
8							+	+				
9				± ?			±				±	
10		±		+, ++			+, +++					
11				++++			++++					
12		− ?		+, ++	++		++		+, ++		±	
13		± ?		+	+		+		+		±	
14				+, ++			++, +++					
15			++	++++								
16			++++ (3½%)	++, +++								
17			++	+++								

Fresh unheated positive defibrinated blood when tested as is heated serum gives strikingly less sensitive results. A simple method of securing satisfactory sensitivity with unheated blood is the addition of an optimal quantity of electrolytes. (See Table III.) Tests of defibrinated blood with various quantities of salt solution and antigen emulsions containing various quantities of cholesterin, showed that results equal to those of the sensitive precipitation test with heated serum followed the use of 0.015 to 0.016 c.c. of 7 per cent sodium chloride solution and 0.017 to 0.018 c.c. of antigen emulsion containing 0.5 c.c. of 1 per cent cholesterin solution in 4.4 c.c. of emulsion.

In the tests with positive unheated defibrinated blood, it was found that the antigen emulsion increases definitely in sensitivity for one-half hour after its preparation, that for the period one-half hour to two hours after its preparation there is no appreciable increase in sensitivity, that after two hours there

TABLE IV

INFLUENCE OF HEAT ON THE SENSITIVITY OF THE ANTIGEN EMULSION

DEFIBRINATED BLOOD NO.	NOT HEATED	ANTIGEN EMULSION HEATED 20 MINUTES TO:			
		42° C.	45° C.	47° C.	50° C.
1		++	+++		
2		±	+		
3		++++	++++		
4		++++	++++		
5		−	−		
6		−	−		
7		−	−		
8		−	−		
9		−	−		
10		−	−		
11	+		++, +++	+++	
12	±, +		++	+++	
13	±, +		+	+, ++	
14	++		++	+++, ++++	
15	++		+++, ++++	+++, ++++	
16	−		−	−	
17	−		−	−	
18	−		−	−	
19	−		−	−	
20	−		−	−	
21	−		−	−	
22	−			±+	+
23	−			±	±+
24				±	±
25	−			±	±
26	±			±+	+
27	±			+, ++	++
28	±			+, ++	++
29	±, +			++, +++	+++
30	+			++, +++	+++
31	++++			++++	++++
32	−			−	−
33	−			−	−
34	−			−	−
35	−			−	−
36	−			−	−
37	−			−	−
38	−			−	−
39	−			−	−
40	−			−	−
41	−			−	−

is again a detectable increase, and that four to six hours after it is made, the emulsion is very sensitive and gives results with positive bloods about equal to those in the very sensitive serum slide tests and in the very sensitive Wassermann test with the same antigen. After reaching its maximum sensitivity in four to six hours, the emulsion retains its antigenic power undiminished for at least twenty-four hours. In negative sera no clumps are formed at any time during this period.

The necessity for waiting four hours or longer for the emulsion to become very sensitive is obviated by heating the emulsion to 50° C. for twenty minutes. The heated emulsion shows much less change on standing than the original sensitive emulsion and may safely be used for at least one and one-half hours. The influence of heat on the sensitivity of the emulsion is shown in Table IV. The quantity of blood (0.04 c.c.) used in the test is sufficient for accuracy and an amount that may be laked (with four times as much distilled water) within a reasonably small space.

The inner small chamber on the slide satisfies the necessity for mixing concentrated ingredients and the outer larger one for subsequently laking the red cells to make possible the reading of the results.

CONCLUSIONS

1. The simple precipitation test for syphilis with a few drops of defibrinated finger blood, described above, is as specific and as sensitive as the microscopic slide precipitation tests and the Wassermann test with the same antigen.

2. Because it requires but a small quantity of comparatively easily obtainable blood, the finger blood precipitation test is particularly useful in the diagnosis of syphilis in infants and children.

3. After its reliability is established by a sufficient number of comparative tests, finger blood may be used routinely in the diagnosis of syphilis.

The development of the finger blood precipitation test was made possible through the cooperation of the Staff of the Out Patient Department of the hospital. Most of the patients tested were referred through the courtesy of Dr. S. Littman from the Department of Dermatology and Syphilology. Dr. B. Levine of this department aided greatly in securing the specimens studied and in evaluating the clinical histories.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LABORATORY TECHNIC

PHENOBARBITAL POISONING: Toxicologic Analysis, Wright, H. N. Arch. Int. Med. 43: No. 1, 85, 1929.

The following method of analysis is proposed:

Hash tissue and extract from three to five times with ether.

Filter

Residue, reserve for further extraction if necessary	Filtrate, concentrate. Contains phenobarbital fats, soaps and some split proteins. Extract with dilute alkali
Ether layer Contains fats	Water layer Contains phenobarbital plus soluble soaps and split proteins. Make acid with acetic acid and extract with ether
Ether layer Contains fairly pure phenobarbital. Repeat extraction if necessary. Identify by melting point 172 to 174 C. and mixed melting point.*	Water layer Contains most of soluble soaps and split proteins.

Colloidal Gold Solutions, Christina, V., and Green, C. S. Psychiatric Quart. July, 1928.

The following reagents and method are recommended:

Conductivity Water: To every liter of distilled water (from a Stokes still) add 1 gm. of chromic acid and distill in an all-glass distilling apparatus, avoiding use of rubber stoppers and connections. To every liter of water distilled over chromic acid add 1 gm. of barium hydrate. This may now be kept as a stock supply to be redistilled immediately before use.

Reagents: Merck's gold chloride acid yellow, 1 per cent solution. Merck's potassium carbonate c.p., 2 per cent solution. Merck's neutral formaldehyde solution, 1 to 40 dilution. These reagents are made up with fresh conductivity water.

Preparation of Colloidal Gold: Heat 1 liter of fresh conductivity water to 60° C.; at this temperature add 10 c.c. of 1 per cent gold chloride solution and 8 c.c. of 2 per cent potassium carbonate solution. Continue the heating until the temperature reaches 92° to 95° C., then remove the flame and add 5 c.c. of formaldehyde solution, 1 to 40 dilution. Stir briskly until a full, bright red color develops.

The necessity of brisk and even stirring must be emphasized, otherwise reduction will not be uniform. After the solution has cooled to room temperature, preferably after standing overnight, the P_H of the gold solution may be determined.

*If any difficulty is experienced in removing soaps, which may be carried over into the ether layer on extraction in acid solution, they may be readily removed by treating the acid aqueous solution with a slight excess of barium chloride solution and filtering off the precipitated barium soaps.

TITRATIONS

The permanent inorganic standards are prepared, with slight modification, according to Taub. The following solutions are necessary for a P_H range of 6.6 to 7.4:

N/2 Cobalt chloride	59.497 gm. $CoCl_2$	$0.6H_2O$ per liter of 1 per cent HCl.
N/2 Ferric chloride	45.054 gm. $FeCl_3$	$0.6H_2O$ per liter of 1 per cent HCl.

The indicator is prepared as follows: Dissolve 1 gm. of phenol red in 52 c.c. of neutral 95 per cent alcohol, add 28.5 c.c. of N/100 NaOH and sufficient conductivity water to bring the volume up to 250 c.c. Store the indicator in a Pyrex bottle.

MATCHING BLENDS

Table I indicates the amounts of chlorides necessary to give the correct colors for the standards.

TABLE I

P_H	CO (C.C.)	FE (C.C.)	CONDUCTIVITY H_2O (C.C.)
6.6	1.8	6.5	11.7
6.7	2.1	5.95	11.95
6.8	2.4	5.4	12.2
6.9	2.95	4.15	12.9
7.0	3.5	2.9	13.6
7.1	4.25	2.1	13.65
7.2	5.0	1.3	13.7
7.3	6.1	0.8	13.1
7.4	7.2	0.3	12.5

The solutions are placed in test tubes of uniform length, diameter and thickness. They are then sealed into ampule form and sterilized in the autoclave at 15 pounds pressure for fifteen minutes to insure complete sterility. The tubes are allowed to cool overnight.

A comparator block is used to make the P_H determination. In making the determinations the test tubes should be of the same diameter and thickness as the uniform tubes used for standards.

To 10 c.c. of the gold solution, add 0.15 c.c. of indicator and mix well. Place this tube in Hole E. In Hole B. place a tube with distilled H_2O. In Holes A, A, place tubes containing gold solution without indicator. Holes C and D are for the P_H standards. If the gold solution does not match the 7.0 P_H standard, then N/100 NaOH or N/100 HCl is added, as the case may require. If the solution shows a P_H below 7.0 alkali is used for the titration; if it is above 7.0 then acid is employed. After each addition of acid or alkali to the gold solution, the contents of the tube are thoroughly mixed by inverting the tube several times.

When the unknown gold solution is matched with the 7.0 standard, the necessary correction is made for the entire volume of gold on hand.

After correction, test the neutrality of the solution by adding 1.7 c.c. of 1 per cent NaCl to 5 c.c. of the colloidal gold. The gold will be completely precipitated in an hour. It must give a typical curve with a known paretic spinal fluid, and no reaction, or a red-blue change at the most in the first or second tube, with a negative spinal fluid.

LEPROSY: A Serological Method of Diagnosis Of, Marchoux, E., and Caro, J. Ann. Inst. Pasteur. 42: No. 5, 542, 1928.

Rubino's red corpuscle sedimentation reaction has been studied by these workers, who obtained positive results in half of ten cases by Ruvino's technic, in which 1 c.c. of the patient's serum is mixed with 1 c.c. of sheep's red corpuscles after washing them in normal saline and adding 10 per cent formal for twenty-four hours and then washing again. On keeping the mixture at 37° C., sedimentation of the red corpuscles within an hour is a positive result. The present workers found that the reaction is a specific one, and that by adding five parts of serum to one of the sheep's corpuscles instead of equal parts, the reaction was positive in all their leprosy cases within half an hour.

In numerous new leper control cases the reaction was never positive. A temperature of 56° C. for an hour destroys the complement of the serum, and one of 60° C. destroys the specific substance as well.

LEPROSY: The Staining of Leprosy Bacilli, Rudel, O. Centralbl. f. Bakteriol. I Abt. Orig. **107:** No. 6, 357, 1928.

The usual TB stain is most successful with leprosy bacilli when the solutions of stains and the sulphuric acid are more or less diluted.

The method is as follows:

(1) Carbol-fuchsin (fuchsin 1.0, alcohol 10.0, concentrated carbolic acid 5.0 to 100.0) with or without ¼ to ½ water; heat until bubbles burst. (2) Wash several times with sulphuric acid (5 per cent) āā water. (3) Wash with water (or in 70 per cent alcohol). (4) Loeffler's methylene blue (concentrated alcoholic methylene blue 30.0, caustic soda solution [0.01 – 100.0] 100.0) āā water, a few seconds up to one minute at the most. (5) Wash with water. (6) Dry and add Canada balsam (or instead of 6: rapidly wash in 70 per cent alcohol for two to five seconds, absolute alcohol one to three seconds toluol balsam).

This stain is so characteristic for leprosy that it can almost be used for differential diagnosis against TB. The leprosy bacilli react much more easily to stains and acids than TB.

TISSUE STAIN: Elastic Tissue Staining, French, R. W. Stain Tech. **4:** No. 1, 11, 1929.

1. Bring sections affixed to the slide into water.

2. Stain in the modified Weigert elastic stain prepared as follows for one-half to three hours:

Crystal violet	2.0 gm.
Dextrin	0.5 gm.
Resorcinol	4.0 gm.
Water, distilled	200.0 ml.

Boil in a porcelain dish. When boiling briskly add 25 ml. of 29 per cent aqueous ferric chloride, stir and continue boiling for 2 to 5 minutes. A heavy precipitate forms and the mixture assumes a greenish cast. Cool and filter. Boil the precipitate in 200 ml. of 95 per cent ethyl alcohol over a water bath or electric hot plate. Cool, filter and make up to 200 ml. Add 4 ml. of concentrated hydrochloric acid.

3. Differentiate in 95 per cent alcohol.

4. Wash in water.

As a routine procedure, preparations are then stained with the Weigert iron hematoxylin or Harris alum hematoxylin and counterstained with the Van Gieson mixture of picric acid and acid fuchsin mixture. Almost any desired stain may be used however, after the elastic stain. The staining is selective, only elastin being stained a brilliant yellowish green.

TISSUE STAIN: Staining of Mitochondria and Bacteria in Plant Tissues, Dufrenoy, J. Stain Tech. **4:** No. 1, 13, 1929.

Milovidov, in Professor Guilliermond's laboratory, has worked out the following technic to stain mitochondria red and bacteria blue in root nodules of legumes.

1. Kill tissues for twenty-four hours in a mixture of 1 per cent chromic acid (50 c.c.), 1 per cent potassium dichromate 50 c.c., 40 per cent formalin (neutralized by adding powdered calcium carbonate) 8 c.c.

2. Rinse twenty-four hours in running water, dehydrate, imbed, and cut very thin sections (4).

3. Dissolve paraffin from slides, dip slides into very thin solution of collodion in absolute alcohol and ether. Run through series of alcohol and water.

4. Stain slides, at temperature of about 80° C. in solution:

Acid fuchsin	2 gm.
Anilin water	10 gm.

5. Wash in running water.

6. Destain for a few seconds in:

Aurantia	0.5 gm.
70 per cent alcohol	100 gm.

7. Wash in water.

8. Treat for a few minutes in:

Phospho-molybdenic acid	1 gm.
Sodium hydroxide, 1 per cent solution	10 gm.
Water	90 gm.

9. Rinse in water.

10. Stain for a few minutes in a solution of Unna's polychrome methylene blue diluted in three parts of water.

STAIN: Staining Methods for Bacteria and Yeasts, Maneval, W. E. Stain Tech. **4:** No. 1, 21, 1929.

For staining flagella of bacteria use actively motile organisms twenty to twenty-four hours old, allow to diffuse in sterile water twenty to thirty minutes, transfer droplets of the suspension to clean slides and let evaporate without spreading. Then treat two to four minutes with the following mordant: tannic acid 10 to 20 per cent, 50 c.c.; ferric chloride 5 per cent, 10 to 15 c.c.; carbol fuchsin (Ziehl-Nielson), 5 c.c.; hydrogen peroxide 3 per cent, 6 to 8 c.c. Wash and stain two to three minutes with a mixture of basic fuchsin, saturated alcoholic, 10 c.c.; anilin oil (1 part) and 95 per cent alcohol (3 parts) mixed; 5 c.c.; distilled water, 30 c.c.; acetic acid 4 per cent, 1 c.c. Wash thoroughly with water.

In some kinds of yeast the nucleus may be demonstrated by fixing smears with heat, staining one minute with aqueous acid fuchsin, followed by 5 per cent tannic acid for twenty seconds and finally washing with acidulated water.

In staining the reaction may be varied by adding acid or alkali to the films and then dropping on the stain. A solution of acetic acid (1:250) or of sodium hydroxide (1:750) is satisfactory. One to four drops of the acid or 1 to 2 drops of alkali may be added to 6 drops of stain. An acid reaction is often the best and sometimes it is well to acidulate slightly the water used for washing.

STAIN: Preparation of Methylene Blue (Loeffler's), Conn, H. J. Stain Tech. **4:** No. 1, 27, 1929.

The keeping qualities of Loeffler's methylene blue stain, when made with commission certified stains, is improved by using the following formula for its preparation:

Methylene blue, medicinal, (90 per cent dye content)	0.3 gm.
Ethyl alcohol, 95 per cent	30 c.c.

Dissolve and dilute in 100 c.c. of distilled water.

TISSUE TECHNIC: The Histological Demonstration of Adrenalin, Baginski, S. Bull. d'Histol. et de Tech. Micr. **5:** 129, 1928.

Former methods for the demonstration of adrenalin in the tissues did not fix the tissues but tended to break them down. The following formulas act as fixatives for the tissue as well as reagents for the detection of adrenalin.

Formula 1.

2 per cent ammonium chromate	30 c.c.
1.25 per cent silver nitrate	20 c.c.
ammonia (0.912 sp. gr.)	3 to 4 drops

Formula 2.

2 per cent ammonium bichromate	30 c.c.
1.25 per cent silver nitrate	20 c.c.
ammonia (0.912 sp. gr.)	7 to 8 drops

These solutions keep well. Fix for three to twenty-four hours according to the size, but pieces should not be larger than 2 to 3 mm. Wash in distilled water until there is no more trace of chromium. Dehydrate slowly, clear in chloroform or xylol, not in cedar oil. Sections may be stained with various dyes. When natural adrenalin is diluted one to ten or fifteen million and is treated with these reagents, a clear test for adrenalin is obtained in the form of a fine black precipitate.

MILK: The Newman Combined Stain. . . For Direct Counting of Bacteria In Milk: Breed, R. S. Am. J. Pub. Health **19**: No. 1, 99, 1929.

The following formula extracts fat, fixes and stains.

FORMULA

Methylene blue powder	1 to 1.12 gm.
Ethyl alcohol, 95 per cent	54 c.c.
Tetrachlorethane, technical	40 c.c.
Acetic acid, glacial	6 c.c.

Add alcohol to the tetrachlorethane in a flask and heat to a temperature not to exceed 70° C. (if it is desired to use methyl alcohol, the temperature should not be raised to more than 55 to 60° C.)

Add the combined solution to the powdered methylene blue. Shake vigorously until the dye is completely dissolved. The original directions state that the glacial acetic acid should be added at this point. It is better to cool the solution before adding acid and the acid should be added very slowly. Further heating of the acid solution should be avoided. Filter and keep in a tightly stoppered bottle.

DIRECTIONS FOR USE

1. Prepare the milk smear.
2. When dry, immerse the smear in the solution and withdraw immediately.
3. Drain until dry (thirty seconds).
4. Wash in water.
5. Dry and observe.

This solution permits much more rapid staining than does No. 1 and yields a clear, well stained preparation. The smears readily wash off the slide unless they are dried before washing (Step No. 3). If dried too rapidly the smears present a spongy appearance. If the smears are dipped in 70 per cent alcohol, this trouble may be prevented.

CL. BOTULINUS: Intramuscular Infection of Guinea Pigs With Spores Of Cl. Botulinus. Coleman, G. E. Am. J. Hyg. **9**: No. 1, 47, 1929.

Coleman calls attention to the fact that animal inoculation with spores of Cl. botulinus and Cl. tetani is uniformly successful with the following technic.

Inoculations of 0.6 to 0.8 c.c. of freshly prepared 10 per cent solution of formalin are made into the leg muscle and a few hours later 1 c.c. of saline spore suspension is injected into the same spot.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Studies in Tristeza**

THIS is a reprint from the Archiv fuer Schiffs- und Tropen-Hygiene and represents a study made on sixteen year and a half old shorthorn steers in Argentina. The steers were infected with a condition known there as Tristeza which corresponds very closely to our Texas Fever. The study of the blood and of the reticuloendothelial system in various organs was quite thorough and it is illustrated with photographs and colored drawings. Some etiologic work and some cross immunity experiments were done and the author inclines to the view that the piroplasma and the anaplasma organisms are distinct because they have been separated as distinct and single infections in cattle.

The Methods Used in the Study of Hemolysis Including Hemagglutination†

THIS is a reprint from Abderhalden's Handbook of Biological Methods and gives a very complete account of the biologic methods by which hemolysis can be produced, the animal and vegetable substances which are hemolytic and the immunologic significance of hemolysis.

A Collection of the Toxic and Lethal Doses for Laboratory Animals of the Most Common Poisons‡

ABOUT 300 poisons are listed beginning with acetanilid and ending with zinc. The data are quite complete as in most cases not only is the dose given but also the animal, the method of administration, the result, and the reference to the original article from which the data were obtained. Such a collection should be very valuable as a general guide or first approximation to the dose but they state that pharmacologists will understand that all such data are more or less variable and should not be used until checked on one's own animals and working with one's own methods and technic.

Combating Loss of Blood by Transfusion and Filling the Vessels§

THIS is one of the Monographic Therapy series and treats the subject in a very practical manner. In the transfusion of homologous blood its scientific basis is discussed, the methods of matching the bloods are given and the technic of the transfusion itself. Then follows a discussion of the indications and the results to be expected.

The clinical justification for filling the blood vessels with fluids other than blood is given, the various fluids that may be so used are described, and their practical application is discussed. He strongly urges the use of Ringer's solution rather than plain physiologic salt solution.

*Studies in Tristeza (Piroplasmosis and Anaplasmosis of Cattle). By Felipe Jimenez de Asua, Roberto L. Dios, Juan A. Zuccarini, and Migues J. Kuhn. Published by Johann Ambrosius Barth, Leipzig, 1927.

†The Methods Used in the Study of Hemolysis Including Hemagglutination. By Hans Sachs and Alfred Klopstock. Published by Urban and Schwarzenberg, Berlin and Vienna, 1928.

‡A Collection of the Toxic and Lethal Doses for Laboratory Animals of the Most Common Poisons. By Flury and Zernik. This is Lieferung 251 of Abderhalden's Handbuch der Biologischen Arbeitsmethoden, Abt. IV, Teil 7, and Heft 7. Pub. by Urban and Schwarzenberg, Berlin, 1928.

§Combating Loss of Blood by Transfusion and Filling the Vessels. By Clairmont, von den Velden, and Wolff. Published by Georg Thieme, Leipzig, 1928.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

*Handbuch der biologischen Arbeitsmethoden**

TWO more parts of this monumental work which is typically German in thoroughness, completeness, and in the excellence of its execution. In no other country could a publisher hope to make his expenses on such an undertaking. Even in rich America the National Research Council had to pass the hat and take up a collection before it could get a publisher for its International Critical Tables. Yet Urban and Schwarzenberg issue this Handbuch along with their regular work and find enough purchasers to make it profitable.

It is this wide-spread spirit of thoroughness that makes Germany such a difficult competitor.

Part 238 contains articles by C. H. Best of Toronto on the Preparation of Insulin and on the Standardization of Insulin. It also contains an elaborate article on the Methods for the Experimental and Histological Examination of the Testis by Schinz and Slotopolsky and one on the Physiological Assay of Cholin by J. W. LeHeux.

Part 235 contains the following articles: The Photomyographic Method of Bethe-Happel by Paul Happel, Methods for the Investigation of and Changes in Irritability and Conductivity in Electrotonus by Otto Weis, Methods for Studying the Physiology of Work with Special Reference to Industrial Fatigue by Edgar Atzler, and Methods for Determining the Distribution of Body Weight on the Different Parts of the Sole of the Foot by Adolph Basler.

Alcohol-Extract-Reaction, A-E-R, for the Diagnosis of Pregnancy, Cancer, and the Sex of the Unborn Child†

THIS is a book of 260 pages on a modification of the Abderhalden reaction. Instead of allowing the patient's serum and tissue to interact the authors mix the serum with an extract of the tissue; instead of dialysing out the digested products the authors remove the undigested proteins by adding 96 per cent alcohol. They claim that these are distinct improvements but admit frankly that they are on their way and have not yet arrived at the goal they have in mind which is to devise diagnostic laboratory tests for many other conditions which will be as reliable as is the Wassermann test in syphilis.

Die Serodiagnose der Tuberculose‡

PINNER gives directions for and discusses agglutination, precipitation, complement fixation, opsonins, the meiostagmin reaction, bacteriolysins, sedimentation of red blood corpuscles, and flocculation reactions as applied to the diagnosis of tuberculosis. He considers complement fixation by far the best specific method but the difficulty with all of them is the inability to distinguish between an active tuberculous process and one that is latent or quiescent.

Proceedings of the Berlin Medical Society for the Year 1926§

THIS is a stately volume of nearly 300 pages and contains a list of their more than 1700 members with addresses, the minutes of their meetings including the annual reports from which it appears that they have a library of 52,000 volumes and take 200 periodicals, and the full text of all of the papers presented at their meetings together with the discussions which followed. Several of the articles are illustrated with special plates.

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*Handbuch der biologischen Arbeitsmethoden, Lieferung 235 and 238.

†Alcohol-Extract-Reaction, A-E-R, for the Diagnosis of Pregnancy, Cancer, and the Sex of the Unborn Child. By W. Luettge and W. von Mertz. S. Hirzel, Leipzig, 1927.

‡Die Serodiagnose der Tuberculose. (Volume 28 of the Library of Tuberculosis being published by Dr. Lydia Rabinowitsch.) By Max Pinner, Director of the State Tuberculosis Sanitorium (Spring Hills Sanitorium) at Northville, Michigan, U. S. A.

§Proceedings of the Berlin Medical Society for the year 1926. Published by the Society, Urban and Schwarzenberg, Berlin.

The Journal of Laboratory and Clinical Medicine

VOL. XIV ST. LOUIS, MO., MAY, 1929 No. 8

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Official Organ of the American Society of Clinical Pathologists and the American Association for the Study of Allergy

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EDITORIALS

Lead in the Treatment of Cancer

THE search for new and better weapons for use in the treatment of disease is indeed a slow and plodding one. Every few years a discovery of the first order is recorded. None would deny a place in this category to insulin or to the liver treatment of pernicious anemia. For each of these outstanding therapeutic advances how many are there which have been hailed as great discoveries but which have not stood the test of prolonged therapeutic trial? How many such have fallen entirely into the discard? How many have temporarily passed into oblivion to be rediscovered after the passage of years and, perhaps with slightly improved technic, have been found after all to possess definite value?

Three years ago the lead treatment of cancer was hailed as possessing great promise. In the interval it has been subjected both to therapeutic trial and to close laboratory experimental study. We are not yet in a position to

assay its proper value but it is interesting after the lapse of three years to survey the progress or lack of progress made.

By a coincidence two communications have appeared almost simultaneously which together bring the literature on the lead treatment of cancer and incidentally our knowledge of the subject up-to-date. The first, a review of the *Present State of the Treatment of Malignant Tumors with Lead* by Jacques Lavedan, Director of Laboratory at the Radium Institute of the University of Paris correlates the available literature. The second, by W. Blair Bell entitled *The Present Position of Lead Therapy in Malignant Disease* summarizes the work of its discoverer up to the present.

Research has been carried on both in the laboratory and in the clinic. In both instances, particularly the second, comparison of results observed by different workers is difficult because in most cases details or even major factors in the technic have varied with nearly each observer. In the clinic, for example, the highly toxic effect of so-called colloidal lead has been recognized and attempts have been made to produce lead compounds of therapeutic potency equal to that claimed for the original used by Blair Bell but with reduced toxicity. As a consequence a relatively large number of compounds of lead have been employed and results with one compound cannot justly be compared with results from another.

In addition many cases have been treated not with lead at all but with a combination of lead and x-ray or lead and radium therapy. Furthermore some have been subjected to prior surgery while others have not and some cases of cancer have been treated while at a relatively early stage of evolution while others have only been subjected to lead therapy if considered hopelessly incurable.

The clinical reports may be roughly divided into two groups, those favorable and those unfavorable. Favorable reports should not necessarily predicate an actual cure in a high percentage of cases but do recognize indisputable benefit attributable to lead therapy in a proportion, and sometimes apparent cure in a few.

Fitzwilliams using a lead preparation different from that originally proposed by Blair Bell has treated 60 cases most of which have also been subjected to surgery. He reports encouraging results especially in several cancers of the esophagus. Carver and Stone have reported results in 21 cases, some treated with lead alone and others with lead and ray therapy. In seven cases of cancer of the breast the tumor diminished markedly in size and in two they felt justified in describing clinical cure. They feel that in osteosarcoma combined lead and ray therapy appears to be the method of choice. Oldham has reported a clinical cure after eighteen months, of a mixed cell sarcoma of the jaw, with glandular metastases. This case was treated with surgery and lead.

Loewy and Loiseleur treated a series of hopeless cancers with a lead preparation of their own with very promising results. When, however, they applied the same therapy to a series of less advanced cases the results were not as good. However, even here 4 out of 21 cases appeared to have been arrested. Thomson applied lead therapy to 55 cancer patients between

October, 1926, and June, 1928. He used three different compounds of lead one of which was the original Bell compound. He found this to be the more effective. Of the 55, fifteen were definitely improved, at least temporarily, of whom 3 have shown no further growth and two are considered cured. Ullman like the others reports cases which have shown no benefit and others which have. Some have been treated with lead alone and others with lead and radiation. Of 15 cancers of the breast 8 showed incontestable improvement of longer or shorter duration. Improvement was also seen in 3 of 4 cancers of the uterus, 2 out of 3 cancers of the maxilla, 2 of 3 cancers of the prostate. Soiland, Costolow and Martland have treated 31 cancers with combined lead and radiation. They described no cures but in 4, while the tumor had not disappeared its further development appeared to be arrested. They feel that they have evidence of at least temporary beneficial effect. However, here as elsewhere the problem arises as to how much is to be attributed to ray therapy and how much to lead.

Knox who has tried both lead therapy and combined lead and ray therapy feels that the latter is of distinctly superior value. He presents a series of 40 cases several of whom showed temporary improvement and 4 of whom may be described as clinically cured. Of course in these clinical cures in all the series mentioned the time interval is too short to say there will be no recurrence.

Finally we have to report among the communications presenting evidence of some beneficial effect from lead treatment the recent communication from Blair Bell mentioned above, covering the results observed in the Liverpool Medical Research Organization. Five hundred and sixty-six cancerous individuals were treated between November 9, 1920, and November 9, 1928. Many of these died before the completion of treatment but of the total, 303 cases received more than one-half of the minimum therapeutic course recommended; 77 died after full treatment; 7 died of intercurrent infection while under treatment; 161 died of the disease before treatment could be completed; 5 died as a result of the extensive destruction from the disease. Twenty-two refused to complete the treatment. In 31, treatment has been completed too recently for the results to be estimated.

Two are believed to have been cured but died from other affections (apoplexy, and toxemia of pregnancy). In 12 the disease has been completely arrested and in 51 the patients are believed cured and the treatment has been discontinued. The last three classifications total 65 cases and Bell records these as successes. He concludes that of 303 cases who received more than half the minimum course advised, 65 or 21.5 per cent resulted successfully.

Opposed to this series of reports we find a sufficiently large number of investigators who can attribute no value to lead therapy. Wyard of the London Cancer Hospital found evidence of benefit in only one of 40 cases treated. This one case presented an enlarged infraclavicular gland following a breast amputation. Under lead treatment the adenopathy disappeared but unfortunately its nature was not definitely known, no biopsy having been made. Wyard believes that lead is entirely without value in the treatment of malignant tumors.

Hume finds no evidence that intravenous injection of lead caused destruction of cancer tissue. Indeed, he feels that this treatment hastened

death. He finds no difference histologically in human malignant tumors, before and after lead injection.

Simpson concludes from his observations that lead does not provide the solution of the therapeutic problem of cancer. Of 19 cases only one has survived and this was a cancer of the breast removed as thoroughly as possible by surgery before the institution of lead therapy.

Waters, Colston, and Gay have treated 7 inoperable carcinomas with combined ray and lead therapy. Five are dead and the sixth, a case of carcinoma of the pleura, improved but developed a pulmonary metastasis, and the seventh with cancer of the breast and metastasis, is improved. In a second series of seven, 3 are definitely improved and 4 are dead. The authors conclude that benefit was attributable in these cases to ray therapy rather than lead since injection of the metal did not prevent the occurrence of metastases.

Leaving for a moment this division of opinion concerning the clinical value of lead, we find some most interesting experimental observations. Mottram finds that in experimental mouse tumor in which the course is quite constant, the lethal dose of lead is accurately known and the minimum curative dose of radium is known, combined radiation and lead treatment produced cures when the dose of each of the two therapeutic measures was distinctly below the minimum curative dose for each used alone. The combination of the two forms of treatment, each insufficient when used alone, resulted in cure. Wood likewise believes that lead and radiation possess synergistic action.

Simpson working with experimental mouse tumors found no curative effect from colloidal lead alone. In these animals at necropsy he was unable to demonstrate lead in the tumor either by microscopic examination or spectroscopic analysis. Bang also found no curative action, indeed not even a perceptible delay in the evolution of the mouse tumor following lead treatment of stated dosage. His dosages were small.

However, as Wood brings out, the fact that lead shows no evidence of therapeutic action on certain animal tumors does not show conclusively that it is not of value in the treatment of human cancer. The evolution of malignant neoplasm is not the same in the two groups.

The observation of Simpson that lead does not accumulate in malignant tumors in experimental animals deserves more attention. It will be recalled that Blair Bell's original concept was that malignant cells possess a very close biologic relationship to the embryonal cells of the chorionic villi, and that just as abortion in lead poisoning is due to a specific affinity between these embryonal cells and the lead, the results of lead in cancer therapy are due to an affinity between the metal and the cancer cells. Simpson found no definite evidence of lead accumulation in mouse tumor. Thomson has found that the spleen and the liver possess a decidedly greater affinity for lead than does the tumor itself.

Soiland and his associates find that colloidal lead does not remain long in the circulating blood. It is quickly phagocytized and becomes fixed in the reticuloendothelial system, accumulating in the bone, liver, and spleen. As long as it remains in a stable state it is held by the histiocytes outside the

tumor. After two or three months it becomes oxidized and hydrolyzed and is eliminated chiefly in the bile. Chemically the major portion is recovered from the spleen, liver, and bone. Both primary and metastatic tumors contain little or none.

Loewy and Loiseleur using oxide of lead which in contrast to the particles of colloidal lead may enter into immediate combination, weighed the lead present in a breast cancer removed forty-eight hours after an injection of one hundred mg. They showed the presence of 0.94 mg. in the 16 gm. of malignant tissue. Here then was actual fixation of lead in the tumor but in only a small fraction of the amount introduced.

Glynn found histologic evidence that lead had influenced the tumor cells in only a small proportion of tumors. Fry concludes again from histologic study that lead produces changes in the convoluted tubules of the kidney and a fatty degeneration of the liver but he was unable to find any evidence of histologic changes in the cancer cells themselves.

Returning for a moment to Bell's theory of resemblance to chorionic cells, it is of interest to note that in chorioepitheliomas, malignant tumors arising from the chorion both Knox, and Stone and Craver were unable to observe any favorable influence following lead treatment.

Apparently lead, if it does exert an action, does not do so by direct effect on the malignant cells. How then does it appear to act?

Lavedan of the Radium Institute of the University of Paris among others confirms the original observation of Blair Bell that lead therapy produces local changes in the tumor such as pain and swelling. If it does not exert a direct action on the malignant cells what then is its *modus operandi?* In Wood's experimental work on rats he observed the same congestion and edema in the tumor. He ascribes it to vascular thrombosis with resulting necrosis. The action then would appear to be vascular with only a secondary necrosis of the tissues and cancer cells. Martland, Sochoky, and Hoffmann also describe peripheral vascular thrombosis with subsequent necrosis of the neoplasm. Lavedan describes a carcinoma of the tongue in which following combined radium and lead therapy the tongue necrosed and sloughed off entirely, without the loss of a drop of blood. Their radium technic was standard and they had had no such experience with radium alone. They interpret this as demonstrating the thrombosing action of lead.

What shall we say, after three or more years of careful study in the hands of many observers, is the present status of the lead treatment of cancer? We can safely say that in a minority of cases there has been evidence of clinical improvement and in a few there appears to have been actual cure. These more or less promising results have almost without exception been obtained in cases which had already advanced to the stage of being considered hopelessly incurable. We can gather from the preceding review that it is not at all certain that cases treated earlier would necessarily show better results. The preponderance of experimental evidence as we have it so far is that lead acts nonspecifically. It acts not through a fatal toxic affinity for cancer cells but by interfering with the circulation of blood and lymph through the tumor thereby strangling the new growth.

The hopeful dream of research workers in cancer is some day to find or to synthesize a substance which will be directly destructive to malignant cells. So far it has not been shown that lead is such a substance. If lead acts only through its capacity to produce thrombosis and if such a reaction should eventually be found to be actually curative in a much larger proportion of cases, the trend of future study on lead and other thrombosing agents will be to find a reagent which acts as efficiently or more efficiently but which does not produce the deleterious side effects of lead poisoning.

Of course such work is now being carried on and as we have said, more combinations of lead have been employed than there have been workers in this field of research. Some of the compounds have been less toxic but most of these have been less potent. The so-called colloidal lead developed by Blair Bell and his associates appears still to be one of the most efficacious if not the most efficacious. But it does damage kidney and liver, produces high grades of anemia and must be avoided where there is any extensive myocardial damage.

Radium and x-ray also appear to act in part at least by interfering with the circulation. The observation that combined ray and lead therapy produces in some hands at least as efficient results as ray therapy alone and with smaller total dosage of the two, appears hopeful.

But the search for a specific will continue. Today we must conclude that from the evidence at hand lead is not a specific. It is of course possible that further studies will eventually demonstrate some truth in Bell's hypothesis that lead acts directly upon the malignant cells. Certainly the work so far is promising enough that investigation will continue. Through the decades before Banting's great work on insulin many men nearly made the discovery. Their reasonings were sound and their experimental work correct as far as it went. But Banting went a bit farther. Ehrlich in his search for a spirocheticide made 606 preparations before he discovered his great specific, salvarsan, and even now we are not quite so certain that salvarsan actually does its work through its specific action as a spirocheticide.

So the search for a specific in cancer will continue. Investigation of the therapeutic value of compounds of lead and other metals will continue at the same time. And we may continue to hope that from one or the other of these two methods of approach the final cure will be developed.

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Lavedan, Jacques: Le Traitement des Tumeurs Malignes par le Plomb. État Actuel de la Question, Paris Med. **19**: No. 11, 272, March 16, 1929.

A detailed bibliography of the names mentioned in this review appeared in Lavedan's article.

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Preliminary Program for Seventh Annual Meeting

Hotel Heathman, Portland, Oregon
July 8 and 9, 1929

Monday Morning Session—9:30 A.M.

1. Presidential Address. Food Allergy as a Cause of Gastrointestinal Symptoms.
   Albert H. Rowe, Oakland, California.
2. Further Clinical Studies and Observations with the New Drug Phenylethanalomine.
   George Piness, Los Angeles, California.
3. A Survey of the Medical Schools of the United States Relative to the Teaching of Allergy.
   Ray M. Balyeat, Oklahoma City, Okla.
4. Bronchial Asthma. The Severe Chronic Intractable Type.
   I. S. Kahn, San Antonio, Texas.
5. Cold Allergy with Systemic Hystamine-like Reactions.
   George E. Brown and B. T. Horton (Guests), Rochester, Minn.

Monday Afternoon Session—1:30 P.M.

1. Hay-Fever—An Historical Sketch.
   Samuel H. Hurwitz, San Francisco, California.
2. Recent Advances in Treatment of Bronchial Asthma.
   Leon Unger, Chicago, Ill.
3. A Survey of Hay-Fever Producing Plants of the Pacific Northwest.
   Robert F. E. Stier, Spokane, Washington.
4. Cooperative Studies in Ragweed Pollen Incidence. Atmospheric Data from Twenty Cities.
   O. C. Durham, Indianapolis, Ind.
5. The Rate of Absorption of Pollen from the Nose.
   Milton B. Cohen, Cleveland, Ohio.
6. Allergy in Its Relation to Bee Sting.
   Robert L. Benson, Portland, Oregon.

Monday Evening—Annual Dinner, 7:30 O'clock

1. Immunological Prophecy from Ancient Hieroglyphics.
   W. Hamilton Manwaring, M.D., Professor of Bacteriology and Experimental Pathology, Stanford University, California.

Tuesday Morning Session—9:30 A.M.

1. Further Use of Digestants in Treatment of Sensitization Phenomena.
   Oliver Harry Brown, Phoenix, Arizona.
2. Cedar Hay Fever, A Clinical Report.
   James H. Black, Dallas, Texas.
3. The Relation of Pulmonary Tuberculosis to Bronchial Asthma.
   Alexander Sterling, Philadelphia, Pa.
4. The Importance of a Two-Year Supervision of Asthma Patients.
   Zella White Stewart, Iowa City, Iowa.
5. Allergy Clinics.

   Robert L. Benson, Portland, Ore.; J. Marr Bisaillon, Portland, Ore.; Ray W. Matson, Portland, Ore.; Ulysses Moore, Portland, Ore.

Business Session

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1927-8

ON TO PORTLAND—EIGHTH ANNUAL CONVENTION

American Society of Clinical Pathologists

AGAIN the members of the American Society of Clinical Pathologists are looking forward with pleasant anticipation to the next meeting of the organization which is to be held in Portland, Oregon, July 5, 6, and 8, 1929. With each succeeding year the Fellows of the Society are being bound more closely together by scientific as well as sentimental ties. The friendships formed and strengthened at the conventions are a source of solace and cheer in the trials and tribulations of the clinical pathologist in his struggle against adverse conditions. The conventions tend to confirm the conviction of the clinical pathologist that our specialty has now become firmly rooted in the medical world, particularly in the hospital field. We find that a better day is dawning for the man who has prepared himself by proper grounding in pathology, bacteriology, biochemistry, and allied sciences and thus made himself indispensable to the clinician both in private as well as institutional practice. Attendance at the conventions is also an inspiration from the scientific standpoint. The papers stimulate the Fellows to better efforts in self-improvement.

The program this year bids fair to equal if not surpass the brilliant successes of the previous years. The next convention marks the eighth mile-

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stone in the existence of our organization which first saw the light of day in St. Louis, in 1922. Little did the pioneers dream that that small band of Charter Members would swell to a membership of now close to four hundred, numbering among them men who have distinguished themselves by original scientific work in the various branches of clinical pathology. A perusal of the tentative program printed on another page shows the diversity of scientific work, almost encyclopedic in its scope, which comes within the purview of the clinical pathologist.

A half day will be devoted to the discussion of a very timely subject in scientific circles, that of undulant fever, on which a number of men who have had experience with this new disease will speak, giving the results of their

MOUNT RAINIER

investigations. There is also a paper on Tularemia by Dr. Walter M. Simpson. Our past president, Dr. Wm. G. Exton, who has never missed a meeting, will give us further information on his studies in the colorimetric methods of determination of urinary constituents. Equally interesting will be further studies by Drs. A. H. Sanford and Charles Sheard on the determination of hemoglobin by a photoelectric method. Those interested in pathologic anatomy and in protozoölogy will likewise find titles of papers which they would like to hear.

The Committee on the Registration of Technicians will have an interesting report to present. Over four hundred laboratory workers throughout the

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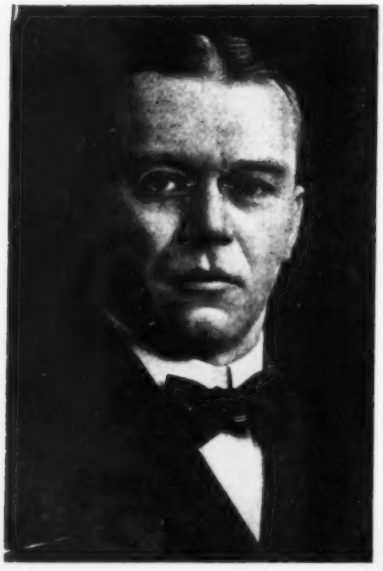

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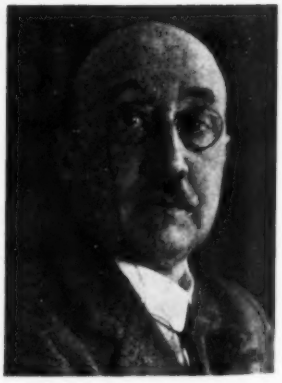

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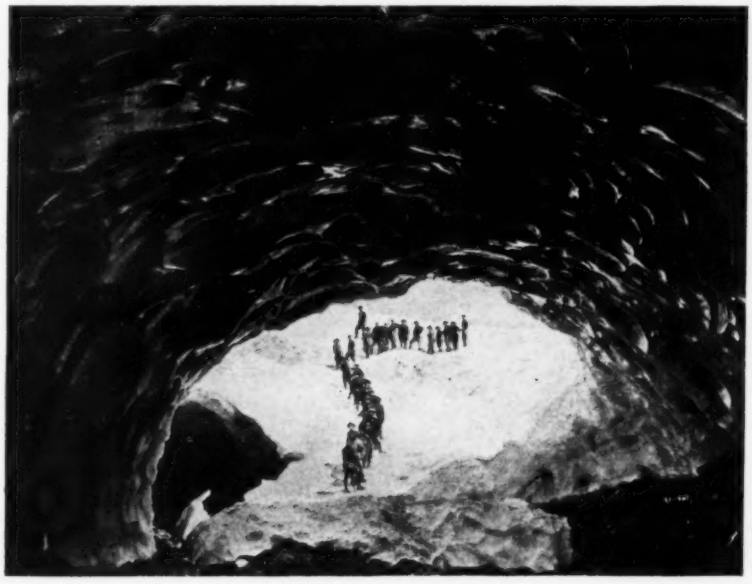
United States and its possessions as well as Canada have enthusiastically endorsed the Registry and have signified their intention to register by filling out the official application for certificates of approval.

One of the features of the meeting is the Round Table Discussion on economic problems where opportunity is given all the members present to let off steam and get the grouch out of their system, receiving in return stimulus and encouragement as well as useful hints from fellow-members. Considerable good has already been effected by these informal discussions. They are a source of great comfort and good fellowship to all the participants.

The place of the Convention on the West Coast need not deter anyone from attending. Aside from a patriotic duty to See America First, the members and their wives will enjoy the outing to the Pacific Coast, which will afford them facilities of viewing the wonderful scenic places at their destina-



Ice Cave in Mt. Rainier National Park

tion and en route. What convention city can be more alluring than Portland, giving an opportunity to visit Mt. Rainier National Park, the unsurpassed scenic beauties of Yellowstone and the Rocky Mountains. The latter are of particular importance, as within their confines is situated the City of Denver where the headquarters of the American Society of Clinical Pathologists is located. The writer succumbs to the temptation of doing a little boosting on his own account by including the Rocky Mountains on the itinerary. A cordial invitation is extended to all the Fellows and their families to visit Denver in their travels, either coming or going.

You are therefore urged to throw all cares aside and rejuvenate yourself with an injection of Western Hospitality and a wonderful vacation by attending the Eighth Annual Convention of the American Society of Clinical Pathologists.

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American Society of Clinical Pathologists

Tentative Program
Of the Eighth Annual Convention

Portland, Oregon

July 5, 6, and 8, 1929

FRIDAY, JULY 5, 1929, 9 A.M.

Scientific Program

The Effect of the Presence of Bile on the Agglutination Reaction. By Ruth Gilbert, M.D., and Marian B. Coleman, B.S., Albany, N. Y.

Tularemia (Francis' Disease): A Report of Ten New Cases with an Analysis of Sixty-Three Cases Occurring in Dayton, Ohio. By Walter M. Simpson, M.D., Dayton, Ohio.

The Routine Use of the Photoelectric Hemoglobinometer. By A. H. Sanford, M.D., and Charles Sheard, Ph.D., Rochester, Minn.

Polycythemia (Patent Foramen Ovale). By Herman Spitz, M.D., Nashville, Tenn.

Acute, Diffuse Myelitis Following Intravenous Injection of Arsphenamine. By Ernest Scott, M.D., and H. L. Reinhart, M.D., Columbus, Ohio.

Improved Colorimetric Procedures for the Quantitative Estimation of the Proteins of the Cerebrospinal Fluid. By Philip B. Matz, M.D., and Nathan Novick, Washington, D. C.

Pachymeningitis Hemorrhagica Interna. By Frederick H. Lamb, M.D., Davenport, Iowa.

FRIDAY, JULY 5, 1929, 2 P.M.

Symposium on Undulant Fever

Undulant Fever in Man: A Clinical Analysis of Thirty-Three Cases. By A. S. Giordano, M.D., and R. L. Sensenich, M.D., South Bend, Ind.

Discussion on Undulant Fever. By Walter M. Simpson, M.D., Dayton, Ohio.

The Pathogenicity for Monkeys of Brucella Abortus. By Merrill J. King, M.D., Mount McGregor, N. Y.

The Etiology and Diagnosis of Undulant Fever in the United States. By Charles M. Carpenter, M.D., and Ruth Book, Ph.D., Ithaca, N. Y.

Bacteriology of Undulant Fever. By K. J. Meyer, M.D., San Francisco, Calif., to be read by J. C. Geiger, M.D., San Francisco, Calif.

FRIDAY, JULY 5, 1929, 7 P.M.

Round Table Discussion

Virtuosity in Clinical Pathology. By Philip Hillkowitz, M.D., Denver, Colorado.

Problems. By W. G. Gamble, Jr., Chicago, Illinois.

The Hospital Situation. (a) Economic, (b) Scientific, (c) Statistical, (d) Relation to American College of Surgeons.

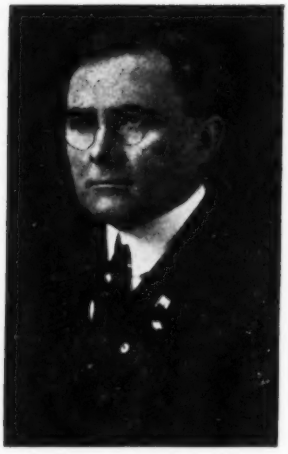
Postmortems.

The Intern Question.

Is the Cost of Laboratory Work Too High?

The Clinical Pathologist in the Rural Hospital.

DR. ERNEST SCOTT
Columbus, Ohio
Board of Censors



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SATURDAY, JULY 6, 1929, 9 A.M.

Gingivitis V: The Character of the Exudate in Gingivitis. By Robert A. Keilty, M.D., Washington, D. C.

The Tuberculous Cavity. By Alfred Blumberg, M.D., Oteen, N. C.

Oxalic Acid as a Reagent for Isolating Tubercle Bacilli and a Study of the Growth of Acid-fast Nonpathogens on Different Mediums with Their Reaction to Chemical Reagents. By H. J. Corper, M.D., and Nao Uyei, Ph.D., Denver, Colorado.

A Recently Isolated Bacillus of the Hemophilic Group. By F. W. Hartman, M.D., and Edna Jackson, M. S., Detroit, Michigan.

Milk Borne Rabies. By E. R. Mugrage, M.D., Denver, Colorado.

Observations on Intestinal Protozoiasis. By Rawson J. Pickard, M.D., San Diego, Calif.

Case of Agranulocytic Angina. By Regina C. Beck, M.D., Richmond, Virginia.

SATURDAY, JULY 6, 1929, 2 P.M.

Pathology of the Reticulo Endothelial System. By Zera E. Bolin, M.D., San Francisco, California.

Reticulocytes: Their Identification and Significance. By C. L. Spohr, M.D., and Alice Bustine, Columbus, Ohio.

Quantitative Microscopic Urinalysis. By Wm. G. Exton, M.D., Newark, N.J.

New Quantitative Clinical Methods for the Junior Scopometer. (1) Protein in Urine, (2) Protein in Blood, (3) Protein in Spinal Fluid, (4) Globulin in Urine, (5) Sugar in Urine, (6) Sugar in Blood, (7) Urea in Urine, (8) Ammonia in Urine, (9) Creatinine in Urine, (10) Sulphur Partition in Urine. By Wm. G. Exton, M.D., Anton R. Rose, Ph.D., Fred Schattner, Ph.D., and P. V. Wells, D.Sc., Newark, N. J. (To be read by title.)

Embryonal Carcinoma of the Testicle. By L. W. Larson, M.D., Bismarck, N. D.

Malignant Tumors of the Testicle. By O. A. Brines, M.D., Detroit, Michigan.

SATURDAY, JULY 6, 1929, 7 P.M.

Annual Banquet

Presidential Address. By Dr. Frank W. Hartman, Detroit, Michigan.

Address. By Dr. Richard B. Dillehunt, Dean of the Medical School of the University of Oregon, Portland, Oregon.

Address. By Dr. Cyrus C. Sturgis, Professor of Medicine at the University of Michigan, Ann Arbor, Michigan.

Presentation of the Ward Burdick Research Award.

MONDAY, JULY 8, 1929, 9 A.M. TO 12 AND 2 P.M. TO 5 P.M.

Business Session

Call to Order.

Reading of Minutes.

Unfinished Business.

Reports of Committees:

Executive Committee—Dr. A. H. Sanford, Chairman, Rochester, Minn.

Publication Committee—Dr. John A. Kolmer, Chairman, Philadelphia, Pa.

Editorial Committee—Dr. T. B. Magath, Editor-in-Chief, Rochester, Minn.

Committee on Registration of Technicians—Dr. Philip Hillkowitz, Chairman, Denver, Colo.

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Committee on Exhibits—Dr. C. H. Manlove, Chairman, Portland, Oregon.

Committee on Necrology—Dr. J. H. Black, Chairman, Dallas, Texas.

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Dr. Edward F. Cooke
Houston, Texas
Chairman, Public Relations Committee

Report of Board of Censors—Election of New Members.
New Business.
Report of Nominating Committee—Nomination of Officers.
Election of Officers.
Induction of Officers.
Adjournment.

Office and Headquarters

The office and headquarters of the American Society of Clinical Pathologists was moved during the year to a downtown location in one of the medical office buildings where the close association with clinicians as well as the facilities of the medical library make the location a very desirable one from many standpoints. We reproduce herewith photographs of the interior with the assistant to the Secretary at her desk.

Office of the Secretary, Front View

An elaborate system of filing devices is part of the equipment, in order to keep in touch with our members as well as to house the data pertaining to the Registry of Technicians.

The map shown in one of the pictures gives a graphic demonstration of the geographic location of our members. The dark pins show the clinical pathologists who are not yet members.

The walls are decorated with photographs of past presidents of the Society.

The new location of our headquarters has added greatly to the efficiency of the Secretariat, being more accessible to the Secretary as well as to the Chairman of the Registry of Technicians, permitting them to spend more time in the office than under the old arrangement in a private hospital. The more prompt mail delivery is also a factor in the increased efficiency. On account of the semipublic character of our work and its benefits to the medical profession the administrators of the building are charging only a nominal rental.

All Clinical Pathologists and licensees of the Registry are invited to visit our office and become personally acquainted. A most cordial invitation is extended to members to stop over on their way to the meeting in Portland to visit our headquarters, where a cheerful welcome awaits them. Facilities will

OFFICE OF THE SECRETARY, SIDE VIEW

be afforded for seeing the wonderful sights of the city as well as the mountains in the environs. The local members will be pleased to extend hospitality to our colleagues and their wives who stop off in Denver going to or coming from the convention.